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Generation effect of Newkome dendrimers on cellular uptake

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ABSTRACT

Poly(amide)-based dendrimers can be used as delivery scaffolds in conjunction with the cell-penetrating peptide gH625 derived from the glycoprotein of the *Herpes Simplex* virus type 1. In this contribution, we aim to isolate the optimal dendrimer generation for cellular uptake for Newkome type dendrimers conjugated with gH625. For this study, we synthesized generations zero to three of the Newkome dendrimer-gH625 bioconjugate. Fluorescent microscopy experiments showed that the second and third generations are the most efficient for cellular uptake with the second generation having the synthetic advantage. The optimal second generation can be used as an improved material for a dendrimer based delivery scaffold for peptide therapeutics.

1. Introduction

Peptide therapeutics have shown promise in various systems but suffer from drawbacks such as protease susceptibility and size limitations common for large therapeutic agents [1,2]. Therapeutics with molecular weights greater than 500 g/mol show potential for the treatment of a variety of diseases ranging from HIV to cancer [3] but delivery of these drugs from the aqueous extracellular matrix across the amphiphilic bilayer of the cell membrane into cells has proven challenging [4,5]. Additionally, peptides are vulnerable to proteases and can lead to immune responses in the body [2]. Conjugation of peptides to polymers can mitigate some of these negatives while allowing for longer circulation times *in vivo* and increased bioavailability [6,7].

One promising class of polymer for peptide ligation for biomedical applications is dendrimers [8–10]. Dendrimer growth is defined by generations, counting each branching point as a new generation [9]. Increasing the number of termini potentially allows for a higher local concentration of drugs either adsorbed in the dendrimer core or attached to the termini. Synthetic complexity, however, also increases with generation [11,12]. Often, higher generation dendrimers are less perfect and have a dispersity above 1. In contrast to linear polymers of the same composition, the radius of gyration of a dendrimer grows linearly with generation while the intrinsic viscosity has a maximum value and then decreases when the dendrimer becomes globular [13]. These properties are an advantage when used in biological applications as increasing the size of the scaffold does not greatly affect the viscosity of the intracellular matrix upon delivery [13]. Branched carriers have been shown to be cleared from the kidneys more slowly than their linear counterparts, resulting in longer circulation times, giving dendrimers another potential advantage [7,14].

Biological systems have been shown to be sensitive to many aspects of polymeric scaffolds. Polymer size, functional density and shape have all been shown to effect cell interactions with polymers [15–17]. Thus, optimization of a polymer-peptide conjugate requires careful study of the polymer's activity in a biological application.

Dendrimer generations *in vivo* have been shown to have a marked effect on the behavior of a dendritic drug delivery scaffold. Different generations often show differences in cell uptake and cell toxicity [18,19]. For example, higher generations of poly(propyleneimine) showed better release of the drug Melphalan, but also a large increase in toxicity [20]. Differences in tumor growth were shown to be negligible between the fourth and fifth generation poly(propyleneimine) dendrimer even as toxicity increased, demonstrating that there is an optimal dendrimer generation for delivery vehicles [20].

The most widely studied dendrimer for delivery applications is poly(aminoamide) (PAMAM), due to its easy availability and low

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cytotoxicity when its cationic nature is mitigated [21,22]. PAMAM dendrimers have been shown to be taken up into cells without the need of cell-penetrating peptides when free amines are present on the termini, however the dendrimer then shows higher toxicity [23]. Obviously, each of the dendrimer systems used as delivery scaffolds for therapeutics, must be optimized for the desired properties [24–26].

The bare dendrimer scaffold is often not enough to deliver cargo into a cell. Building on previous work, our strategy to increase cellular uptake of the scaffold takes advantage of the dendrimer scaffold ligated to the peptide gH625 (Fig. 1), derived from a segment of the glycoprotein H from Herpes Simplex virus type 1 [27]. This peptide sequence is able to enter the cell and deliver various cargos, a proposed mechanism suggests the amphiphilic nature of gH625's α helical architecture allows the interaction with cellular membranes [1,27,28] (see Fig. 2).

We have previously demonstrated that compared to free gH625, attachment to the termini of a second generation (G2) Newkome-type dendrimer scaffold greatly increases cellular uptake with low cell toxicity up to 20 μM [29,30]. At that time, we also performed cell viability assays to determine the optimal concentration of peptidodendrimer based on the concentration of peptide using UV–vis analysis. This concentration was used in our study to allow for comparison between these findings and previously reported studies.

The uptake was previously measured using both fluorescence microscopy and flow cytometry and it was shown in both cases that the peptidodendrimer had an advantage over free gH625 [29,30]. The use of the G2 dendrimer was wholly arbitrary in our prior research; no comparison was made between various generations of dendrimer. The initial results suggested a potentially new peptide scaffold based on Newkome-type dendrimers functionalized with gH625 as a cell penetrating peptide to deliver payload into HeLa and Vero cells. As a first step towards the optimization of our cell penetrating scaffold, this contribution investigates the generation dependence of our gH625 functionalized Newkome-type delivery scaffold to determine which generation is most suited for further study. We show that there is an ideal size for our scaffold based on cellular uptake, ease of synthesis and maximization of cargo.

2. Results

Our dendrimers of choice are poly(amide)-based with a 1 → 3 branching unit structurally derived from dendrimers first reported by Newkome, Scheme 1 [31,32]. All dendrimers are synthesized from the commercially available bifunctional dendrons di-*t*-butyl-4-[2-(*t*-butoxycarbonyl) ethyl]-4-aminoheptanedicarboxylate (aminotriester) and 4-(2-carboxyethyl)-4-nitroheptanedioic acid (nitrotriacid). The dendrons are coupled using carbodiimide/DIPEA peptide-coupling schemes. The nitro group is reduced subsequently to an amine in order to yield a reactive terminus. The tert-butyl esters are hydrolyzed yielding multiple reactive termini. This strategy can be repeated multiple times to yield the dendrimer of the desired generation (Fig. 1). A 3-azidopropylamine linker can be coupled to each terminus of the dendrimer of interest giving a handle to attach alkyne-functionalized peptides using copper catalyzed 1,3-dipolar cycloaddition [33–35]. Alternatively, a dendrimer can be directly functionalized using peptide bond coupling strategies. All dendrons are synthesized using iterations of the coupling and deprotecting reactions and detailed syntheses and

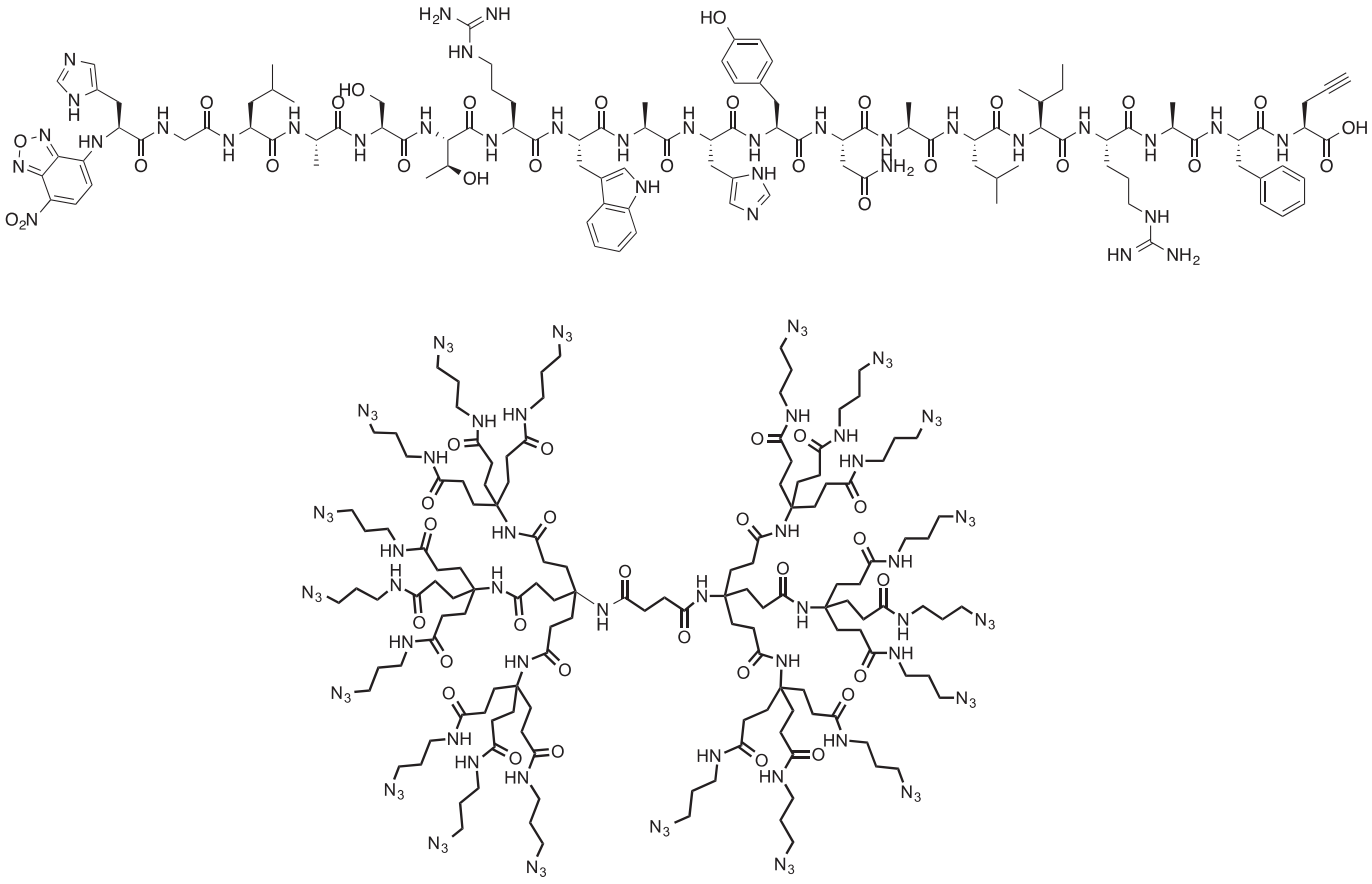

Fig. 1. Previously synthesized second generation azidodendrimer and structure of dye-alkyne modified gH625.