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Nitric oxide-releasing hyperbranched polyaminoglycosides for antibacterial therapy

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Abstract

Hyperbranched polyaminoglycosides were prepared by the polymerization of kanamycin, gentamicin, and neomycin, and *N,N'*-methylenebis(acrylamide) via a one-pot reaction. The secondary amines at the linear units of the hyperbranched polymers were subsequently reacted with NO gas at high pressure under alkaline conditions to form *N*-diazoniumdiolate NO donors. The resulting NO-releasing hyperbranched polyaminoglycosides exhibited a wide range of NO payloads (~ 0.4 – $1.3 \mu\text{mol mg}^{-1}$) and release kinetics (half-lives ~ 70 – 180 min). The therapeutic utility of these materials was evaluated by examining their bactericidal activity against common dental pathogens and toxicity to human gingival fibroblast cells. The antibacterial efficacy of NO-releasing hyperbranched polyaminoglycosides was dependent on specific physiochemical properties, with greater degrees of branching and aminoglycoside terminal groups correlating to enhanced action. Nitric oxide-releasing hyperbranched polykanamycin and polyneomycin elicited the least cytotoxicity at bactericidal concentrations, indicating the greatest therapeutic index for future biomedical applications.

Graphical Abstract:

Keywords

Nitric oxide; Hyperbranched polyaminoglycosides; Antibacterial; Dental pathogens

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ASSOCIATED CONTENT

Supporting Information Experimental details, NMR spectra, FTIR spectra, and UV-vis spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare the following competing financial interest(s): Mark H. Schoenfisch is a co-founder and maintains a financial interest in Novan, Inc. and Vast Therapeutics, Inc. Both companies are commercializing macromolecular nitric oxide storage and release vehicles for clinical indications.

Introduction

Dental caries and periodontitis are among the most prevalent diseases in humans.¹ Dental caries (i.e. tooth decay) affect 60 – 70% of school-aged children and a majority of adults in most industrialized countries.² Roughly, 11% of the world's population suffers from severe periodontitis that contributes to tooth loss and systematic health defects such as coronary diseases, cardiovascular diseases, stroke, and adverse pregnancy outcomes.^{3–5} Of >700 microorganisms in the oral cavity, the overgrowth of cariogenic bacteria (e.g., *Streptococcus mutans*) and periodontal pathogens (e.g., *Porphyromonas gingivalis*) drives the initiation and progression of these oral diseases.^{6–9} Developing oral therapeutics capable of killing these disease-causing bacteria is thus important to maintain a healthy oral cavity.^{10–12}

Nitric oxide (NO), a free radical produced endogenously, plays an important role in the natural immune response. The antibacterial activity of NO stems from its ability to exert nitrosative or oxidative stress through its reactive byproducts (e.g., peroxynitrite and dinitrogen trioxide), ultimately disrupting the integrity of bacterial membrane and compromising cell function.¹³ Due to multiple killing mechanisms decreasing the likelihood of bacteria fostering resistance, research focused on the development of NO-based therapeutics has been intense.^{14–20} Equally important is that NO has proven effective against a number of antibiotic-resistant bacteria, representing another advantage of its use for antibacterial therapy.^{15, 21–22} Our group has previously synthesized NO-releasing polyamidoamine (PAMAM) dendrimers, and evaluated their antibacterial action against dental bacteria. Although these materials were effective against Gram-negative periodontal pathogens, they were not capable of eradicating Gram-positive cariogenic bacteria at safe concentrations (i.e., non-toxic toward mammalian cells).^{23–24} In addition, the synthesis of dendrimers is tedious and time consuming because of the need for multistep purification.

Hyperbranched polymers are attractive alternatives to traditional dendrimers as a result of their straightforward synthesis and comparable properties (e.g., unique three-dimensional dendritic shape, and high density of functional groups).^{25–27} Recently, we demonstrated the utility of hyperbranched polymers (e.g., PAMAM polymers and polyesters) as NO-delivery scaffolds.^{28–29} Despite having greater architectural defects, NO-releasing hyperbranched PAMAM polymers exhibited comparable bactericidal efficacy and cytotoxicity to its structurally perfect G3-PAMAM dendrimer counterparts. However, the synthetic cost of hyperbranched PAMAM is significantly lower than that of dendrimers.

Hyperbranched polyaminoglycosides represent another class of hyperbranched polymers with inherently attractive features. First, they can be readily prepared via a one-pot polymerization of aminoglycosides (naturally occurring antibiotics) and diacrylates or diepoxides.^{30–33} These materials have been shown to exhibit favorable biodegradability and low toxicity resulting from many glycosidic linkages and hydroxyl groups, respectively. As common antibiotics, aminoglycosides possess broad-spectrum antibacterial action.³⁴ The high density of aminoglycosides therefore conferred high antibacterial activity to the hyperbranched polyaminoglycosides. Indeed, previous studies have demonstrated the efficacy of polyaminoglycosides against *Escherichia coli* and *Staphylococcus aureus*.^{31–33}

Herein, we describe the synthesis and characterization of NO-releasing hyperbranched polyaminoglycosides conjugated from various naturally produced aminoglycosides (i.e., kanamycin, gentamicin, and neomycin). The use of specific exterior functional groups is studied with respect to NO-release and antibacterial properties. Based on a previous study describing synergistic effects of co-delivering aminoglycoside (i.e., gentamicin) and NO using a block copolymer system against both planktonic and biofilm cultures of *P. aeruginosa*,³⁵ we evaluated the hypothesis that the combination of inherently antibacterial hyperbranched polymers (i.e., hyperbranched polyaminoglycosides) with NO release may enable a potent acting therapeutic that is able to eradicate both Gram-positive and Gram-negative dental pathogens without eliciting unwarranted toxicity to healthy mammalian cells.

Experimental Section

Synthesis of hyperbranched polyaminoglycosides.

Hyperbranched polyaminoglycosides were prepared according to previous reports.^{30–31} In a typical synthesis, 2.50 mmol of a given aminoglycoside (KA, NE, or GE) sulfate was mixed with 3.75 mmol of Bis-MBA in distilled water (50 mL). This solution was supplemented with sodium bicarbonate at an equivalent molar ratio to the sulfates combined with the aminoglycosides. The reaction mixture was stirred for 3 d under nitrogen atmosphere at 60 °C to yield hyperbranched polyaminoglycosides (i.e., HPKA, HPNE, or HPGE). The resulting solution was concentrated by rotary evaporation and subsequently dialyzed against distilled water for 3 d with a dialysis tubing (MWCO 2000). To obtain HPKA with various exterior functional groups, 2.50 mmol of KA were first mixed with 6.25 mmol of Bis-MBA in 50 mL of distilled water supplemented with sodium bicarbonate (5.00 mmol) and reacted for 3 d at 50 °C under a nitrogen atmosphere. Next, 0.50 mL of EDA or MEA was added as the capping agent into the reaction mixture and allowed to react for an additional day at 40 °C to obtain HPKA-EDA or HPKA-MEA, followed by the same dialysis procedure. The purified products were recovered by lyophilization as a powder and stored at 4 °C until further use. Hyperbranched polyaminoglycosides were characterized by nuclear magnetic resonance (NMR) spectrometry. Representative ¹H NMR data of HPKA contained the following peaks (400 MHz, D₂O, δ): 1.0–1.5 (CHCH₂CH); 2.2–3.3 (O=CCH₂CH₂, O=CCH₂CH₂, NCH, CHNH, CHNH₂, CHCH₂NH₂, CHCH₂NH, CHCH₂N), 3.3–3.8 (CH₂OH), 4.4 (NHCH₂NH), 5.0–6.0 (CH(OCH)₂CH). Representative ¹H NMR data of HPKA-MEA consisted of the following peaks: 1.0–1.5 (CHCH₂CH); 2.2–3.3 (O=CCH₂CH₂, O=CCH₂CH₂, NCH, CHNH, CHNH₂, CHCH₂N, CH₂CH₂OH), 3.3–3.8 (CH₂OH), 4.4 (NHCH₂NH), 5.0–6.0 (CH(OCH)₂CH).

Synthesis of *N*-diazoniumdiolate-modified NO-releasing polymers.

The addition of *N*-diazoniumdiolate NO donors onto water-soluble scaffolds was carried out according to previous reports.^{36–37} Hyperbranched polyaminoglycosides (20 mg) were dissolved in a mixture of MeOH (0.2 mL) and water (0.8 mL) combined with a 25 μL of 5.4 M sodium methoxide (one molar equivalent relative to total amine content of the polymer, as determined by elemental analysis). The reactor was briefly purged with argon three times, followed by three longer purges (10 min) with argon to remove oxygen. The reactor was

then filled with 10 atm NO. The pressure was maintained during the formation of *N*-diazoniumdiolate NO donors. After 3 d, the reactor was flushed with argon again using the same procedure to remove the unreacted NO. The product (i.e., HPKA/NO, HPNE/NO, HPGE/NO, HPKA-EDA/NO, and HPKA-MEA/NO,) was precipitated out by acetone, washed with methanol, and dried under vacuum. The products were stored at -20°C until further use.

Statistics.

Differences between the NO payloads and NO-release kinetics of NO-releasing hyperbranched polyaminoglycosides were analyzed using one-way ANOVA analysis, and $p < 0.05$ was considered as statistical significance.

Additional Information.

Characterization of nitric oxide release, in vitro planktonic bactericidal assays against dental pathogens (i.e., *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans*, *Actinomyces viscosus*), confocal fluorescence microscopy experiments, and cytotoxicity assays against human gingival fibroblasts were carried out according to previous reports.^{21, 29} The details are reproduced in the Supporting Information file.

Results and Discussions

Hyperbranched polyaminoglycosides (HPA) were synthesized via one-pot reactions as described by Chen et al. (Scheme 1).^{30–31} Naturally-occurring aminoglycosides (kanamycin, neomycin, or gentamicin) were reacted with *N,N'*-methylenebisacrylamide (Bis-MBA) at a molar ratio of 2:3 to yield aminoglycoside-terminated HPA polymers (HPKA, HPNE, or HPGE, respectively). As terminal groups are reported to affect both nitric oxide (NO)-release properties and antibacterial action of the modified materials,^{21, 38–39} hyperbranched polykanamycin, as a proof of concept system, was modified with various terminal groups (Scheme 2). Specifically, the molar ratio of kanamycin and Bis-MBA was increased to 2:5, yielding a vinyl-terminated intermediate,³¹ and subsequently reacted with ethylenediamine (EDA) or mono-ethanolamine (MEA) to produce HPKA terminated by EDA (HPKA-EDA) or MEA (HPKA-MEA), respectively.

The molecular weight and polydispersity index (PDI) of the HPA polymers were measured by size exclusion chromatography instrument configured with a multiangle light scattering detector (Table 1). The molecular weight for each HPA polymer was dependent on the aminoglycoside identity, likely due to the different reactivity of each aminoglycoside. FTIR spectra revealed adsorption bands located at ~ 2930 and $\sim 2840\text{ cm}^{-1}$, assignable to $-\text{CH}_2$ and $-\text{CH}_3$ stretching vibrations. The bands at $\sim 1650\text{ cm}^{-1}$ and $\sim 1530\text{ cm}^{-1}$ were assigned to carbonyl stretching of Bis-MBA and amino bending vibration of aminoglycosides, respectively, suggesting successful polymerization from aminoglycoside and Bis-MBA monomers (Figure S1). ^1H NMR analysis indicated the disappearance of peaks (5.6–6.6 ppm) from the double bonds of Bis-MBA and the appearance of broad peaks from the newly formed ethylene ($-\text{CH}_2-\text{CH}_2-$) bonds (2.2–3.0 ppm), further confirming the polymerization

via Michael-addition reactions (Figure S2). As a typical hyperbranched polymer, HPA consists of dendritic (D), linear (L), and terminal (T) units.^{25, 27, 40} The appearance of several peaks between 25–60 ppm in the ¹³C NMR spectra was attributed to ethylene groups under different chemical environments. The ethylene groups assignments were adapted from previous reports, with results provided in Figure S3.^{30–31} The degree of branching (DB) was estimated based on the following equation: $DB = 2D / (2D+L)$.^{27, 40} The DBs of HPA, HPNE, and HPGE ranged from 0.32 to 0.58 (Table 1), indicating the formation of highly branched structures.

Synthesis of *N*-diazoniumdiolate-modified NO-releasing polysaccharides.

Macromolecular scaffolds utilized for NO-release applications, such as polyamidoamine (PAMAM) dendrimers and chitosan, often require an additional modification step to convert primary amines to secondary amines prior to NO loading as NO loads preferentially and more efficiently at secondary amine sites.^{36, 41–42} Hyperbranched polyaminoglycosides, in contrast, can be directly modified with *N*-diazoniumdiolates due to their abundant secondary amines present on the linear units (Figure S3). Hyperbranched polyaminoglycosides were reacted with NO gas at high pressure (10 atm) under alkaline conditions, yielding *N*-diazoniumdiolate NO donor-functionalized HPA polymers (HPKA/NO, HPNE/NO, HPGE/NO, HPA-EDA/NO, and HPA-MEA/NO). The representative UV-vis characterization is provided in Figure S4. Control (i.e., non-NO-releasing) polymers exhibited an absorbance peak at ~290 nm, attributable to the protonated tertiary amines from the dendritic units.⁴³ After NO loading, a characteristic *N*-diazoniumdiolate NO donor peak at ~250 nm is observed, confirming successful formation of *N*-diazoniumdiolates.⁴⁴

The NO-release properties for the NO donor-modified HPA polymers were measured using a chemiluminescence nitric oxide analyzer (NOA) in PBS (pH 7.4) at 37 °C. The total NO payloads and NO-release kinetics of aminoglycoside-terminated HPA polymers (HPKA/NO, HPNE/NO, and HPGE/NO) were found to be dependent on the aminoglycoside identity. This result may be attributed to the differences in amine content as shown in Table 1. The aminoglycoside-terminated HPA polymers with larger amine content exhibited greater NO payloads, as a result of more readily available reactive sites for *N*-diazoniumdiolate formation. The longer half-life was also associated with the higher amine contents, likely due to the formation of intramolecular hydrogen bonds between neighboring cationic amines and *N*-diazoniumdiolate anions, stabilizing the diazoniumdiolates and slowing decomposition.^{39, 45}

Altering the terminal groups of HPA from KA to EDA or MEA led to an increase in NO payloads (Figure 1A). Indeed, the NO-release levels from HPA-EDA/NO and HPA-MEA/NO (~1.20 μmol mg⁻¹) were three times greater than that of HPA/NO (~0.40 μmol mg⁻¹), even though their amine content was only slightly greater (Table 1). Additionally, a fraction of the greater nitrogen content for HPA-EDA is a result of more primary amines, that are not able to form stable *N*-diazoniumdiolates.⁴¹ The difference in NO payloads is thus attributed to the location of the secondary amines, which affects the reactivity of amines with NO. For HPA, the secondary amines present on the linear units would be more randomly distributed along the polymer backbone (Scheme 1). The interior secondary

amines may have limited access to both the base and NO gas, hampering their conversion to diazeniumdiolates.⁴⁶ In contrast, the synthesis of HPA-EDA and HPA-MEA results in the secondary amines concentrating at the exterior of the polymer and enhances *N*-diazeniumdiolate NO donor formation efficiency (Scheme 2). The identity of exterior functional groups also influenced the NO-release kinetics (Figure 1B). HPA-EDA/NO ($t_{1/2}$ ~185 min) exhibited more extended NO release relative to HPA-MEA/NO ($t_{1/2}$ ~74 min) (Table 2), as the formation of hydrogen bondings between the primary amines of EDA and the diazeniumdiolates likely stabilized the NO donors.

Bactericidal study against planktonic dental pathogens.

The bactericidal action of NO-releasing and non-NO-releasing (control) HPA polymers was evaluated against common bacteria associated with dental diseases, including the Gram-negative *P. gingivalis* and *A. actinomycetemcomitans*, and the Gram-positive *A. viscosus* and *S. mutans*. The bactericidal assay was performed under static conditions in 1 vol% broth-supplemented PBS (pH 7.4, 37 °C). The minimum bactericidal concentration (MBC_{2h}) required to achieve a 3-log (99.9%) reduction in bacterial viability after a 2 h exposure was used to quantify the bactericidal activity of the HPA polymers. The NO dose ($\mu\text{g mL}^{-1}$) was derived by multiplying the amount of NO released over the 2 h exposure time (i.e., $t[\text{NO}]_{2h} \times \text{molar mass of NO}$) and the corresponding MBC_{2h} value.

As shown in Tables 3 and 4, control (i.e., non-NO-releasing) HPA polymers only exhibited moderate antibacterial efficacy against dental pathogens, as evidenced by their large MBC values. The addition of NO release significantly improved the antibacterial action of the HPA polymers, demonstrating NO as the key bactericidal agent. Further inspection of the MBC_{2h} values and NO dose revealed that NO treatment was more effective against Gram-negative bacteria (*P. gingivalis* and *A. actinomycetemcomitans*) compared to the Gram-positive microbes (*S. mutans* and *A. viscosus*). Such differential bactericidal activity to NO has previously been attributed to the thicker peptidoglycan cell membranes of Gram-positive bacteria.^{21, 23, 47}

The antibacterial action of the NO-releasing HPA polymers proved highly dependent on the aminoglycoside identity (Table 3 and Table 4). Although $t[\text{NO}]_{2h}$ ($\sim 0.25 \mu\text{mol mg}^{-1}$) was comparable for each system (Table 2), HPNE/NO exhibited the strongest antibacterial action, followed by HPA/NO, and HPGE/NO the least effective. The discrepancies in bactericidal action are attributed to the differences in the polymer structures as represented by their degrees of branching (DBs). For example, the HPA polymers with greater DBs (0.58, 0.49, and 0.32 for HPNE, HPA, and HPGE, respectively) exhibited stronger antibacterial activity. It has been well documented that the spatial structure of hyperbranched polymers becomes more compact as DB increases, resulting in a greater density of functional groups.^{48–50} Therefore, the NO-releasing HPA polymers with greater DBs are rationalized to have enhanced *N*-diazeniumdiolate NO donor density, potentially enabling more efficient NO delivery to the bacteria with concomitant bactericidal action.^{13, 16, 51} Of note, the MBC_{2h} values of HPNE/NO and HPA/NO were significantly lower (i.e., MBC_{2h} 8 mg mL^{-1}) than that of NO-releasing G1-PAMAM dendrimers and silica nanoparticles (i.e., MBC_{2h} 48 mg mL^{-1}).²³ Compared to recently reported NO-releasing hyperbranched

PAMAM systems (i.e., NO dose $120 \mu\text{g mL}^{-1}$),²⁹ HPNE/NO and HPKA/NO required lower NO doses (i.e., NO dose $60 \mu\text{g mL}^{-1}$) to achieve the same killing, indicating the advantages of these NO-releasing hyperbranched polyaminoglycosides.

Upon modifying the terminal groups of HPKA with EDA or MEA, a decrease in bactericidal action was observed, as evidenced by the increased NO dose required to kill bacteria (Table 3 and Table 4). In addition, HPKA-EDA/NO and HPKA-MEA/NO were observed to have comparable bactericidal efficacy. Given their similar polymer structures as indicated by the DBs (Table 1), the terminal groups are likely the pivotal factor in determining the antibacterial activity of the NO-releasing HPKA systems. Confocal fluorescence microscopy was used to elucidate the killing mechanism of bactericidal action. Intracellular NO accumulation and cell membrane damage was visualized using DAF-2 DA and PI fluorescence probes, respectively (Figure 2).^{21, 47, 52} Negligible autofluorescence signal was found in the *S. mutans* bacterial solution. After exposing *S. mutans* to HPKA/NO, an initial intracellular NO accumulation was observed at 30 min, followed by the appearance of cell membrane damage and depletion of the accumulated NO (beginning at 60 min). Exposure of *S. mutans* to an equivalent concentration of HPKA-MEA/NO only led to the occurrence of intracellular NO with minimal cell membrane damage. The confocal fluorescence data suggest that the kanamycin terminal groups of HPKA/NO contribute to more efficient cell membrane damage relative to other terminal groups (i.e., MEA or EDA).

In vitro cytotoxicity of hyperbranched polyaminoglycosides.

The toxicity against healthy mammalian cells is an important factor when evaluating the therapeutic potential of a new antibacterial agent. The toxicity of both NO-releasing and control HPA polymers was evaluated using human gingival fibroblasts (HGF-1) over a 2 h exposure period. For control HPA polymers, HPNE exhibited the most pronounced toxicity, while HPGE exhibited the lowest toxicity. The EDA and MEA terminal groups mitigated toxicity at concentrations 8 mg mL^{-1} relative to KA-terminated HPKA, indicating that aminoglycoside terminal groups may adversely affect mammalian cells at these concentrations (Figure 3). Indeed, Hu et al. reported that aminoglycosides have limited killing selectivity against bacteria relative mammalian cells.⁵³ Consistent with previous reports,^{23, 29, 54} the NO-releasing HPA polymers usually exhibited greater toxicity to HGF-1 than control polymers (Figure 3B), especially at high concentrations (8 mg mL^{-1}). The increased toxicity is expected because large doses of NO are known to induce apoptosis-mediated mammalian cell death.⁵⁵ Nevertheless, HPKA/NO and HPNE/NO did not elicit significant toxicity (i.e., $\sim 50\%$ cell viability) to HGF-1 at their highest effective bactericidal concentrations (8 and 4 mg mL^{-1} for HPKA/NO and HPNE/NO, respectively), which is advantageous over previous NO-releasing G1-PAMAM dendrimers and silica nanoparticles, and comparable to hyperbranched PAMAM polymers.^{23, 29} Further studies including preclinical periodontitis models are currently underway to further ascertain the therapeutic potential of the NO-releasing HPA polymers.

Conclusion

Nitric oxide-releasing hyperbranched polyaminoglycosides with diverse NO payloads (~ 0.4 – $1.3 \mu\text{mol mg}^{-1}$) and release kinetics (half-lives ~ 70 – 180 min) were prepared by modifying secondary amines within the hyperbranched polymers with *N*-diazoniumdiolate NO donors. The NO release significantly improved the antibacterial activity of the hyperbranched polyaminoglycosides against common bacteria linked to oral diseases. Polymers with greater degrees of branching and aminoglycoside terminal groups (i.e., kanamycin) proved to be more effective at eradicating the bacteria. In particular, HPKA/NO and HPNE/NO were capable of eradicating the dental pathogens at concentrations that did not compromise the viability of healthy human gingival fibroblast cells. Experiments are underway to evaluate the antimicrobial activity of these materials against multi-species dental plaque biofilms and in a preclinical model of infection to further understand the therapeutic potential of NO-releasing hyperbranched polyaminoglycosides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A): Cumulative and (B) real-time nitric oxide-release profiles for the first 5 h for HPA/NO (black), HPA-EDA/NO (red), HPA-MEA/NO (green).

Figure 2. Confocal fluorescence images for visualizing the real-time antimicrobial behavior of (A) HPKA/NO (0.1 mg mL⁻¹) and (B) HPKA-MEA/NO (0.1 mg mL⁻¹) against *S. mutans*. Green fluorescence represents DAF while red fluorescence is PI. Scale bar = 20 μm.

Figure 3. Percent cell viability of human gingival fibroblasts (HGF-1) following a 2 h exposure to: (A) control and (B) NO-releasing hyperbranched polyaminoglycosides.

Scheme 1.

Synthesis of aminoglycoside-terminated hyperbranched polyaminoglycosides.

Scheme 2.

Synthesis of ethylenediamine (EDA)- or monoethanol amine (MEA)-terminated hyperbranched polyaminoglycosides.

Table 1.

Characterization of hyperbranched polyaminoglycosides.

Polysaccharide	M_n^a (g mol ⁻¹)	M_w^a (g mol ⁻¹)	PDI ^a	DB ^b	Nitrogen ^c (wt%)
HPKA	2.96×10^3	5.86×10^3	1.98	0.49	10.71
HPNE	1.63×10^4	2.07×10^4	1.27	0.58	12.23
HPGE	2.35×10^4	3.92×10^4	1.67	0.32	14.21
HPKA-EDA	2.25×10^3	4.31×10^3	1.92	0.45	15.18
HPKA-MEA	4.74×10^3	6.69×10^3	1.41	0.46	12.70

^a M_n (number average molecular weight), M_w (weight average molecular weight), and polydispersity index (PDI) were measured by SEC-MALS characterization.

^b DB (degree of branching) was estimated from quantitative ¹³C NMR.

^c Nitrogen (wt%) was determined by elemental analysis.

Table 2.

Nitric oxide-release characterization for polysaccharides in 10 mM PBS (pH 7.4) at 37 °C.^a

Polysaccharide	t[NO] ($\mu\text{mol mg}^{-1}$) ^b	t[NO] _{2h} ($\mu\text{mol mg}^{-1}$) ^c	t _{1/2} (min) ^d
HPKA/NO	0.40 $\pm$ 0.08	0.24 $\pm$ 0.07	70 $\pm$ 12
HPNE/NO	0.53 $\pm$ 0.12	0.29 $\pm$ 0.08	103 $\pm$ 33
HPGE/NO	0.60 $\pm$ 0.14	0.25 $\pm$ 0.07	147 $\pm$ 23
HPKA-EDA/NO	1.20 $\pm$ 0.21	0.46 $\pm$ 0.07	185 $\pm$ 25
HPKA-MEA/NO	1.28 $\pm$ 0.28	0.77 $\pm$ 0.17	74 $\pm$ 21

^aEach parameter was analyzed with n = 3 separate syntheses

^bTotal NO payloads per milligram polysaccharides

^cThe amount of NO released after the initial 2h

^dTime to release half of total NO payloads.

Table 3.

The minimum bactericidal concentration (MBC_{2h}) and the corresponding NO dose of hyperbranched polyaminoglycosides against Gram-negative dental bacteria.^a

Polysaccharides	<i>P. gingivalis</i>		<i>A. actinomycetemcomitans</i>	
	MBC _{2h} (mg mL ⁻¹)	NO dose (μg mL ⁻¹)	MBC _{2h} (mg mL ⁻¹)	NO dose (μg mL ⁻¹)
HPKA	16		16	
HPKA/NO	2	14	1	7
HPNE	8		8	
HPNE/NO	0.5	5	0.5	5
HPGE	>16		16	
HPGE/NO	4	30	2	30
HPKA-EDA	>16		16	
HPKA-EDA/NO	4	55	2	28
HPKA-MEA	>16		16	
HPKA-MEA/NO	2	46	1	23

^a
n = 3 replicates

Table 4.

The minimum bactericidal concentration (MBC_{2h}) and the corresponding NO dose of hyperbranched polyaminoglycosides against Gram-positive dental bacteria.^a

Polysaccharides	<i>S. mutans</i>		<i>A. viscosus</i>	
	MBC _{2h} (mg mL ⁻¹)	NO dose (μg mL ⁻¹)	MBC _{2h} (mg mL ⁻¹)	NO dose (μg mL ⁻¹)
HPKA	>16		>16	
HPKA/NO	8	55	2	14
HPNE	>16		8	
HPNE/NO	4	35	1	9
HPGE	>16		>16	
HPGE/NO	>16	>120	4	30
HPKA-EDA	>16		16	
HPKA-EDA/NO	16	221	4	55
HPKA-MEA	>16		>16	
HPKA-MEA/NO	8	185	4	92

^a
n = 3 replicates