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Antimicrobial Efficacy of Synthesized Quaternary Ammonium Polyamidoamine Dendrimers and Dendritic Polymer Network

C. K. V. Zainul Abid[1], Richa Jackeray[1], Swati Jain[1], Sruti Chattopadhyay[1], S. Asif[2], and Harpal Singh[1,*]

[1] *Center for Biomedical Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India*
[2] *Glycoprotein Research Lab., National Institute of Immunology, New Delhi 110067, India*

Water treatment to mitigate microbial contaminants is a major challenge across globe paving the way to develop novel antimicrobial compounds. We aim at architecting antibacterial moiety eventually catering to vast water treatment industry. In this research study, quaternary ammonium functionalized polyamidoamine (PAMAM) dendrimer and PAMAM-ethyleneglycol dimethacrylate (EGDMA) dendritic polymer network were synthesized. These materials were characterized by various analytical techniques like ATR-FTIR, 1HNMR, DSC etc. Water soluble generation (G) 1.0 PAMAM dendrimer and water insoluble PAMAM G1.0 EGDMA dendritic polymer network were quaternized by reacting with dilute hydrochloric acid (HCl) and octyl iodide (OI) respectively. Both quaternary ammonium dendrimer products were found to exhibit potent bactericidal activity against a group of common Gram-negative and Gram-positive bacteria. 10 mg/L concentration of liquid PAMAM G1.0 QHCl was efficient to kill 100% bacteria rapidly within an incubation time of just 2 minutes. In addition, quaternary ammonium dendritic polymer network PAMAM G1.0-EGDMA Q OI demonstrated good contact killing antimicrobial property without releasing any active molecule into the surrounding medium and disinfected contaminated water within 5 minutes. Both quaternary ammonium dendrimer and dendritic polymer network showed negligible cytotoxicity in MTT assay indicating their potential as a viable antimicrobial agent.

Keywords: Chemical Synthesis, Polymers, Nanostructures, Biomaterials.

1. INTRODUCTION

Microbial infection has been a serious issue throughout the history of human existence on earth. Researchers all over the world are working towards developing various chemical agents which can destroy infectious microorganisms.[1–3] There are several types of antimicrobial agents available in market, but the toxicity, short life period and the fact that microbes are always evolving with formation of highly resistant strains have been a serious issue of concern.[4,5] Increased hurdles have prompted closure scrutiny of such disinfectants and preferential development of novel non-toxic antimicrobials. Polymeric disinfectants are a step forward in this direction due to their lower toxicity and non-irritant properties with prolonged period of action as compared with that of ordinary disinfectants like phenols, halogens, ferrocene, boron derivates, small quaternary ammonium compounds (QACs) etc.[6,7] Polymeric quaternary ammonium compounds have garnered popularity due to their stable and non-toxic nature.[1,3–5,8–10] Antimicrobial action of the QACs is based on their surfactant like electrostatic damaging interaction with the cytoplasmic membrane of the microorganism, resulting in loss of membrane permeability. At convenient optimum concentrations, they cause cell leakage and eventually cell death.[6] Literature suggests that the polymers containing QACs, not only enhance efficacy over corresponding small QAC molecule, but also show increased efficiency and selectivity, and prolonged lifetime.[1,4,11,12]

Recently a new class of material, dendrimer, has garnered attention as possible antimicrobial agent due to their compact structure and their ability to accommodate large number of end functional groups.[13] Dendrimers

*Author to whom correspondence should be addressed.

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are novel highly branched three dimensional cascading macromolecules of nanodimension that radiate from a central core.[14, 15] Since molecular size and generation of dendrimers are increased stepwise via the repetition of reaction sequence, their size and structure are highly controllable.[14, 16, 17] Multiple peripheral functionality incur synergistic and combinatorial interactions with other molecules.[18] Polyamidoamines (PAMAM) are the first and extensively studied dendrimer for various biomedical applications due to their nanoscopic structure and non-immunogenic nature.[19–21] Lee et al.[22] have quaternized G4.0 PAMAM dendrimers with methyl iodide for gene delivery applications and observed that cationic PAMAM dendrimers were efficient in complexing with plasmid DNA and showed good cellular membrane penetration efficiency with lower cytotoxicity. Modified PAMAM dendrimer has been studied for anti-tumor drugs such as methotrexate, 5-flurouracil etc.[23, 24] Amino terminated fourth generation of PAMAM has been shown to complex with folic acid using PEG4000 as a spacer, effectively encapsulates anticancer drug and demonstrated better pharmacokinetic, dynamic and biodistribution efficacy as compared with naked drug molecules while treating mice induced with cancer.[25] Recently Ghosh et al.[26] have reported the quaternization of G 3.0 PAMAM dendrimer with dimethyl dodecyl amine after modification with 2-chloroethyl isocynate to produce antimicrobial textile fabrics. These treated fabrics showed effective results against *Staphylococus aureus* bacteria on antimicrobial studies. PAMAM has been known to deliver antimicrobial drugs[27] to cells but their effect on bacteria is largely uncharted. In one report, PAMAM derivatives have been investigated as potential antimicrobial agents against gram negative bacteria especially *Pseudomonas aeruginosa* with 0.9–1.5 μg/mL as EC_{50}.[28]

In the present study, we have synthesized water soluble PAMAM G1.0 dendrimer and water insoluble poly(PAMAM G1.0 EGDMA) dendritic polymer network operating on simple chemistries without employing multistep reactions for generating secondary antibacterial polycations. These liquid and solid dendrimers were quaternized and evaluated for antimicrobial activity to be used as sterilizing and disinfecting agent respectively.

2. MATERIALS AND REAGENTS

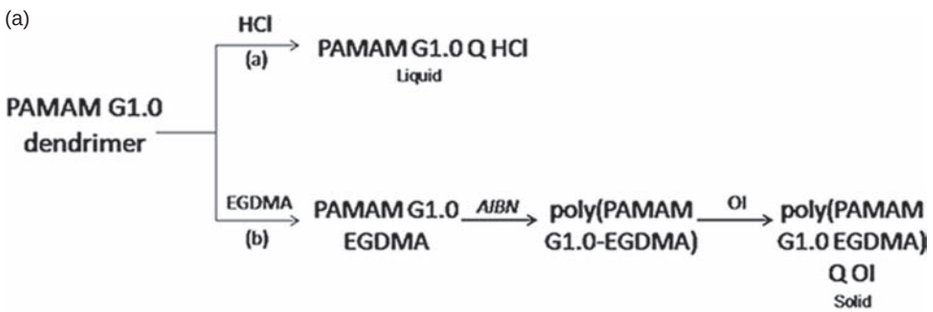
Methyl acrylate (MA), ethylene glycol dimethacrylate (EGDMA) and methylthiazolyldiphenyl-tetrazolium bromide (MTT) reagent were obtained from Sigma-Aldrich (St. Louis, USA). Ethylene diamine (EDA), hydrochloric acid (HCl), octyl iodide (OI), dimethyl sulphoxide (DMSO) and azobisisobutyronitrile (AIBN) were purchased from CDH chemicals (New Delhi, India). HPLC grade methanol (MeOH) and toluene were obtained from Loba Chemicals (Mumbai, India). Luria broth, nutrient agar, bacterial strains-*Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 33807), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633) were obtained from Hi-Media Laboratories (Mumbai, India). All the reagents and chemicals were used as received unless noted otherwise.

3. EXPERIMENTAL DETAILS

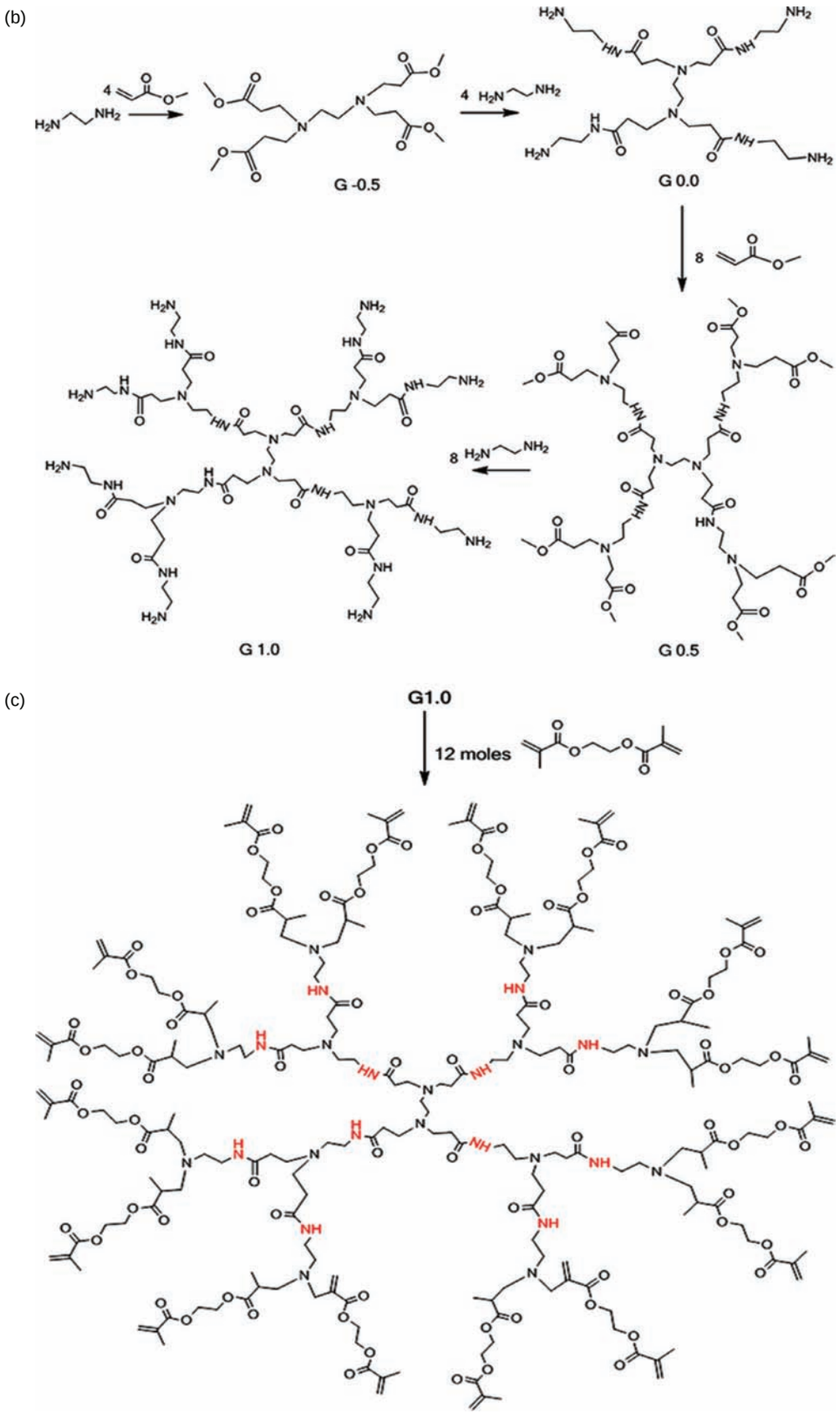
3.1. Synthesis and Quaternization of Dendrimers

3.1.1. PAMAM G1.0 Dendrimer and Quaternization

Synthesis of PAMAM dendrimers was carried out following a two step process, involving Michael addition of ethylene diamine initiator core with methyl acrylate and exhaustive amidation of resulting esters with large excess of ethylene diamine as reported in literature.[16, 20, 29, 30] In brief, 80% w/v freshly distilled EDA was reacted with 80% w/v solution of methyl acrylate in methanol in 1:50 molar ratio for 24 h at room temperature under nitrogen blanket. The resultant product was purified and was called G −0.5 dendrimer. Further, the solution of G −0.5 dendrimer in methanol (80% w/v) was carefully added to a vigorously stirred solution of EDA in methanol (80% w/v) in 1:50 molar ratio at 0 °C and reaction was continued for 96 hours at room temperature to obtain G 0.0 dendrimer with amine end functional groups. Above steps were repeated with G0.0 dendrimer to obtain G1.0 dendrimer as shown in Scheme 1. All reactions were carried



Scheme 1. Continued.



Scheme 1. (a) Flowchart depicting steps for synthesis of quaternized dendrimers (b) Chemical reactions representing synthesis of different generations PAMAM dendrimers (c) Synthesis of PAMAM G1.0-EGDMA dendrimer.

out in amber colored round bottom (RB) flask and the temperature was never allowed to increase above 30 °C to avoid undesirable side reactions due to light and heat initiation. All addition reaction steps were followed by the removal of excess reagents and solvents by rotary vacuum evaporator at temperature below 35 °C.

Synthesized PAMAM G1.0 (1 mole) and 3 N HCl (2.5 mole) were taken in RB flask equipped with a magnetic stirrer. Temperature of the system was adjusted to 40 °C and reaction was allowed to proceed for 6 h. Excess of HCl and water were removed by rotary vacuum evaporator and the liquid product was stored in amber coloured bottle for further characterization and use.

3.1.2. PAMAM G1.0-EGDMA Dendrimer

Methanolic solution of PAMAM G1.0 (80% w/v) (Section 3.1.1) was added to a stirred solution of ethyleneglycol dimethacrylate (EGDMA) in methanol (80% w/v) in 1:20 mole ratio, under nitrogen, over a period of 1 h at 0 °C and continuously stirred for 24 h at room temperature (Scheme 1). Solvent and excess of EGDMA were removed by rotary vacuum evaporator at 40 °C. Resulting pale yellow oil was further vacuum dried overnight (10–1 mm Hg, 50 °C) to remove any traces of methanol and EGDMA (Yield = 98%).

3.1.3. Dendritic Polymer Network Poly(PAMAM G1.0-EGDMA) and Quaternization

PAMAM G1.0-EGDMA was polymerized by free-radical polymerization technique using azo bis-isobutyronitrile (AIBN) as free-radical initiator. PAMAM G 1.0-EGDMA and AIBN were vortexed in a glass test tube (0.06 w/w of total dendrimer) to form a uniform solution. Glass test tube was kept on a water bath at 75 °C for 10 min to get solid transparent polymer(poly(PAMAM G1.0-EGDMA). Synthesized product was kept at 70 °C for 1 h for complete curing and was later on washed several times with hot distilled water to remove any traces of unreacted dendrimer/initiator from the system.

4 g of poly(PAMAM G1.0-EGDMA) was crushed into small pieces and was refluxed at 40 °C with 30 mL octyl iodide for 6 h in a round bottom flask with constant stirring to quaternize amino groups present in the polymer. This quaternized product poly(PAMAM G1.0-EGDMA) Q OI was washed several times with hot distilled water to remove excess of octyl iodide and was dried under vacuum.

3.2. Characterization

The structures of both dendrimers and dendritic polymeric network were extensively studied with attenuated total reflectance-Fourier transform infra red (ATR-FTIR) spectroscopy and spectra were recorded on Perkin-Elmer spectrum one spectrometer attached with single bounce diamond ATR accessory. Bruker AC 300 spectrometer of 300 MHz frequency was used for recording 1HNMR spectrum of synthesized compounds in $CDCl_3$ solvent. Deuterated dimethyl sulhpoxide (DMSO) was used as solvent to record the nuclear magnetic resonance (1HNMR) spectrum of PAMAM G1.0 and PAMAM G1.0 Q HCl. Differential scanning calorimetry (DSC) studies were carried out using Perkin Elmer DSC-6 system. Vacuum-dried samples were loaded into calorimeteric system and thermograms were obtained in the temperature range of −50 °C to 200 °C under nitrogen atmosphere at the heating rate of 10 °C/min. ZEISS EVO 50 scanning electron microscope (SEM) was employed to study surface morphology of synthesized dendritic polymeric networks. Samples were dried under vacuum overnight and were mounted on a metallic stub and images were taken after coating the stub with silver to provide conduction for better resolution of images. Solid quaternized PAMAM G1.0 dendritic network of EGDMA were also thoroughly tested for its gel content, swelling behavior and leaching parameters.

Gel content of the quaternized dendritic polymer was determined by immersing the dry and weighed amount (1 g) of polymer in chloroform at room temperature for 24 h. Sample was then taken out, dried at 60 °C and weight of the sample was noted. Gel content was calculated using the following equation.

$$\text{Gel Content } (\%) = \left[W_i - \left(\frac{W_i - W_f}{W_i} \right) \right] \times 100$$

where, W_i and W_f are the weights of the dried polymer before and after immersing in chloroform respectively.

Swelling studies of the dendritic polymer networks are important because of their future use as water disinfectant and dendritic polymer should not contaminate the water upon degradation due to excessive swelling. Water absorption capacity of the quaternized dendritic polymer was determined by immersing the dry polymer sample (1 g) in distilled water. The sample was taken out from water at various time intervals and weighed after blotting out excess water from the surface of polymers with filter paper for 10 seconds. They were put back in water immediately after weighing. The percent water absorption of the synthesized polymer was calculated using the following equation.

$$\text{Percentage swelling } (\%) = \left[\frac{(W_s - W_d)}{W_d} \right] \times 100$$

where, W_s and W_d are the weight of the polymer in the swollen and dry states respectively. The experiment was repeated thrice for each specimen and average values are reported.

To evaluate the leaching property of the prepared dendritic polymer network 2 g of dried quaternized dendritic polymer network was kept in 10 mL distilled water for a period of 30 days and UV-Visible absorption of the water was tested regularly with a five days interval using

10 mm path length transmission micro probe connected to a Cary 50 UV-Visible spectrophotometer (Spectral range 190–1100 nm) wavelength. After 30 days polymer was taken out, dried in vacuum oven and the weight of polymer was noted.

3.3. Antimicrobial Activity of PAMAM G1.0 QHCl and Poly(PAMAM G1.0-EGDMA) QOI

The antibacterial activity of liquid PAMAM G1.0 QHCl and solid poly(PAMAM G1.0 EGDMA) QOI were evaluated against *E coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* common Gram-negative and Gram-positive bacteria by colony count method.[31] 10 mL of all bacterial cultures (10^8 CFU/mL) were prepared in separate test tubes. 0.05 and 0.1 mg of PAMAM G1.0 QHCl and poly(PAMAM G1.0 EGDMA) QOI were added in 10 mL of bacterial contaminated water to obtain 5 mg/L and 10 mg/L concentration for a fixed incubation time (2 min, 5 min and 10 min) under constant shaking. Then 100 μL aliquots were withdrawn from the above water samples and laid over the nutrient agar plates using sterile glass spreader and incubated for 24 h at 37 °C. All experiments were done in triplicate and negative control contained no quaternary ammonium compound. Log reduction values of bacteria for all quaternary ammonium compounds were calculated after two minutes of incubation time using following equation.[32]

$$\text{Log}_{10}\text{reduction} = (\text{Log}_{10}\text{initial bacterial count}) - (\text{Log}_{10}\text{final bacterial count})$$

3.4. Evaluation of Antimicrobial Efficiency of Poly(PAMAM G1.0 EGDMA) QOI for Repetitive Use

0.1 mg of quaternary ammonium dendritic polymer, poly(PAMAM G1.0 EGDMA QOI), was kept in contact with 10 mL of bacterial solution (10^8 CFU/mL) for 5 minutes (final concentration being 10 mg/L). 100 μL aliquot was withdrawn from this solution and laid over nutrient agar plate using sterile glass spreader. Rest of the solutions was completely removed and fresh 10 mL of bacterial solution (10^8 CFU/mL) was added. Same procedure was followed for twenty five cycles. All plates were incubated for 24 h at 37 °C along with a negative control and the growth of bacteria was examined by colony count method.

3.5. Evaluation of MIC and MBC of PAMAM G1.0 Q OI

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. It is a basic laboratory practice to measure the activity of antimicrobial agent against microbe and to monitor the resistance of microorganisms to an antimicrobial agent.[33] The minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial drug required to kill an organism. Antimicrobials are usually regarded as bactericidal if the MBC is not more than four times of the MIC.[11,34] Different concentrations (ranging from 200 mg/L to 1.25 mg/L) of PAMAM G1.0 Q OI were prepared in autoclaved Luria broth solution and 1 mL of each concentration was transferred to glass test tubes. To each test tube, 100 μL of 10^8 CFU/mL bacterial solution was added and incubated for 24 h at 37 °C. After this, test tubes were checked for the formation of turbidity. Turbid test tubes indicated the growth of bacteria. 100 μL was withdrawn from each non turbid test tube and plated to find out number of colonies formed. MIC was taken as the amount of QAC at which no turbidity formation or increase in initial bacterial concentration was observed. MBC was taken as the minimum concentration of quaternary ammonium dendrimer at which there was no turbidity and a 100% killing of test bacteria was observed.

3.6. *In Vitro* Toxicity Assessment of PAMAM G1.0 QHCl and Poly(PAMAM G1.0 EGDMA) QOI by MTT Assay

MTT stock solution of 12 mM concentration was prepared by dissolving 5 mg of MTT reagent in 1 mL of PBS buffer. 1.5 mg of each ammonium dendrimer and dendritic polymer was added to wells of 96 well plate in triplicate and 100 μL of Jurkat cell (T-lymphocyte) suspension with a cell density of 10^6 cells/mL were added into each well and the plate was incubated at 37 °C for 24 h. To each well, 10 μL of the 12 mM MTT stock solution was added and incubated at 37 °C for 3 h. MTT reagent gets reduced into purple colored formazan crystals if metabolic activities (viability) are present in the cell population. 50 μL of DMSO was added to each well to solubilize formazan crystals formed and the contents of the wells were centrifuged separately to remove all insoluble materials. After collecting 100 μl supernatant to fresh wells, absorbance was recorded on UV spectrophotometer at 540 nm.[35] All experiments were done in triplicate, a negative control where no dendritic samples added to cell suspension with 10 μL MTT solution and a positive control with 10 μL of the MTT stock solution added to 100 μL of medium were also included in the experiment.

4. RESULTS AND DISCUSSION

4.1. Synthesis and Quaternization of Dendrimers and Dendritic Network

Two amino groups of EDA react with four acrylate groups by Michael addition generating acrylate terminated structure designated as G −0.5 dendrimer as illustrated in Schemes 1(a) and (b). These end groups further underwent exhaustive amidation with excess of EDA, generating G 0.0 dendrimer with amino groups at the ends. Subsequent repetition of these two steps yielded hyperbranched yellow colored liquid of G1.0 dendrimer of around 100 nm

size and having 14 quaternizable nitrogen moieties. As seen from Scheme 1, primary and secondary amines of PAMAM G1.0 were completely quaterized with acid HCl yielding water soluble PAMAM QHCl dendrimer. Typically, excess of acids was used to facilitate the reaction and to prevent intradendrimer quaternization.

The two reactive protons of primary amines of PAMAM G1.0 converted to dimethacrylate moieties on reaction with EGDMA having terminal double bonds which were polymerized with AIBN to yield a highly intricate transparent solid compound as represented in Scheme 1(c). Amine groups of its core dendritic structure reacted with long hydrocarbon salt octyl iodide resulting in quaternary ammonium solid derivative. Octyl iodide was chosen since it has long hydrocarbon chain which significantly improves antimicrobial activities of QACs.[36]

4.2. Characterization of Synthesized Dendrimers and Network

4.2.1. ATR-FTIR Spectroscopy

The IR spectrum of PAMAM G1.0 and quaternized product, PAMAM G1.0 Q HCl are given as Figures 1(a) and (b). A broad peak at 2952 cm^{-1} and increase in the intensity at 3244 cm^{-1} is attributed to the formation of quaternary ammonium (+NH) group. Michael addition reaction of PAMAM G1.0 with EGDMA was confirmed by the appearance of –C═C peak at 810 cm^{-1} and ester –C═O peak at 1722 cm^{-1} as seen from Figure 2(a). Figure 2(b) shows the IR spectrum of poly(PAMAM G1.0-EGDMA) in which absence of –C═C peak at 810 cm^{-1} confirmed the complete utilization of double bonds in polymerization reaction. Quaternized polymer (Fig. 2(c)) clearly showed the presence of –CH stretching of octyl iodide at 2956 cm^{-1} and 2923 cm^{-1} confirming the quaternization of poly(PAMAM G1.0 EGDMA) to form poly(PAMAM G1.0 EGDMA) Q OI.

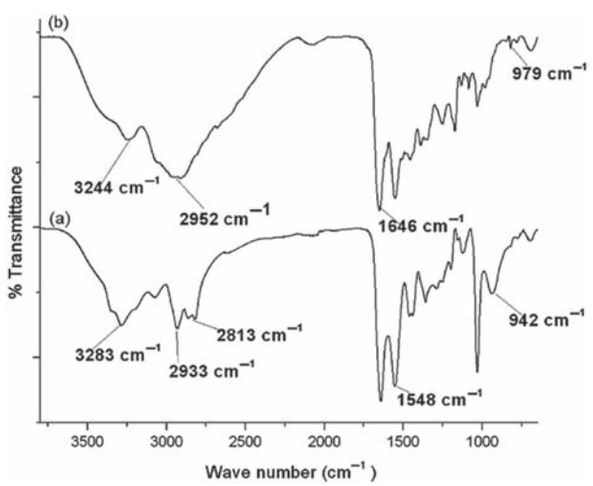


Figure 1. FTIR spectrum of (a) PAMAM G1.0 (b) PAMAM G1.0 Q HCl.

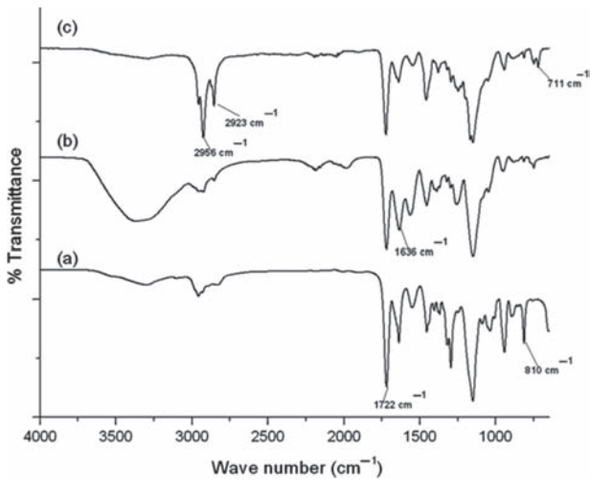


Figure 2. FTIR spectrum of (a) PAMAM G1.0 EGDMA (b) poly(PAMAM G1.0 EGDMA) (c) poly(PAMAM G1.0 EGDMA) Q OI.

4.2.2. NMR Spectroscopy

1HNMR spectrum of PAMAM G 0.5 is given as Figure 3(a). Formation of ester terminated product is confirmed by the appearance of –O–**CH_3** peak at 3.6 ppm (24 H, s). A peak corresponding to –CO–NH–**CH_2**–CH_2–N– at 3.25 ppm (8 H, q) further confirmed the formation of ester terminated product PAMAM G 0.5. Absence of –O–**CH_3** peak at 3.6 ppm and presence of **–NH_2** peak at 2.45 ppm (16 H, t) confirmed the formation of PAMAM G1.0 in Figure 3(b). The expanded 1HNMR spectra of (c) PAMAM G1.0 and (d) PAMAM G1.0 QHCl proved quaternization of dendrimer as new peaks at 8.50–8.64 ppm appeared. Michael addition reaction of PAMAM G1.0 with EGDMA was confirmed through the appearance of vinyl proton peak at 5.59 ppm and 6.13 ppm (16 H, s) as seen from Figure 3(e). Other characteristic peaks of the new compound are as follows: 4.40 ppm (64 H, s, –N–CH_2- C(CH_3)–CO–O–**CH_2–CH_2**–O–CO–C(CH_3)═CH_2); 1.94 ppm (48 H, s, –N–CH_2–C(CH_3)–CO–O–CH_2–CH_2–O–CO–C(**CH_3**)═CH_2); 1.19 ppm (48 H,d, –N–CH_2–C(**CH_3**)–CO–OCH_2–CH_2–O–CO–C(CH_3)═CH_2); 3.29 ppm (24 H, –CO–NH–**CH_2**–CH_2–N–); 3.6 ppm (8 H, t, –(**CH_2–CH_2**)$_2$–N–CH_2–CH_2–N-(**CH_2–CH_2**)$_2$-).

4.2.3. Differential Scanning Calorimetry

The DSC thermograms of solid PAMAM G1.0-EGDMA and PAMAM G1.0-EGDMA QOI are shown as Figure 4. Shifts of the glass temperature (Tg) determined from the thermograms are considered as a reliable criterion for the assessment of corresponding polymeric structure after modifications. *Tg* of PAMAM G1.0-EGDMA was 138 °C where as its quaternized version showed a slightly higher *Tg* of 146 °C due to increase in charge density with the incorporation of octyl iodide. Lengthy inflexible chain of octyl group also decreased the dendritic mobility which leads to increased *Tg*.

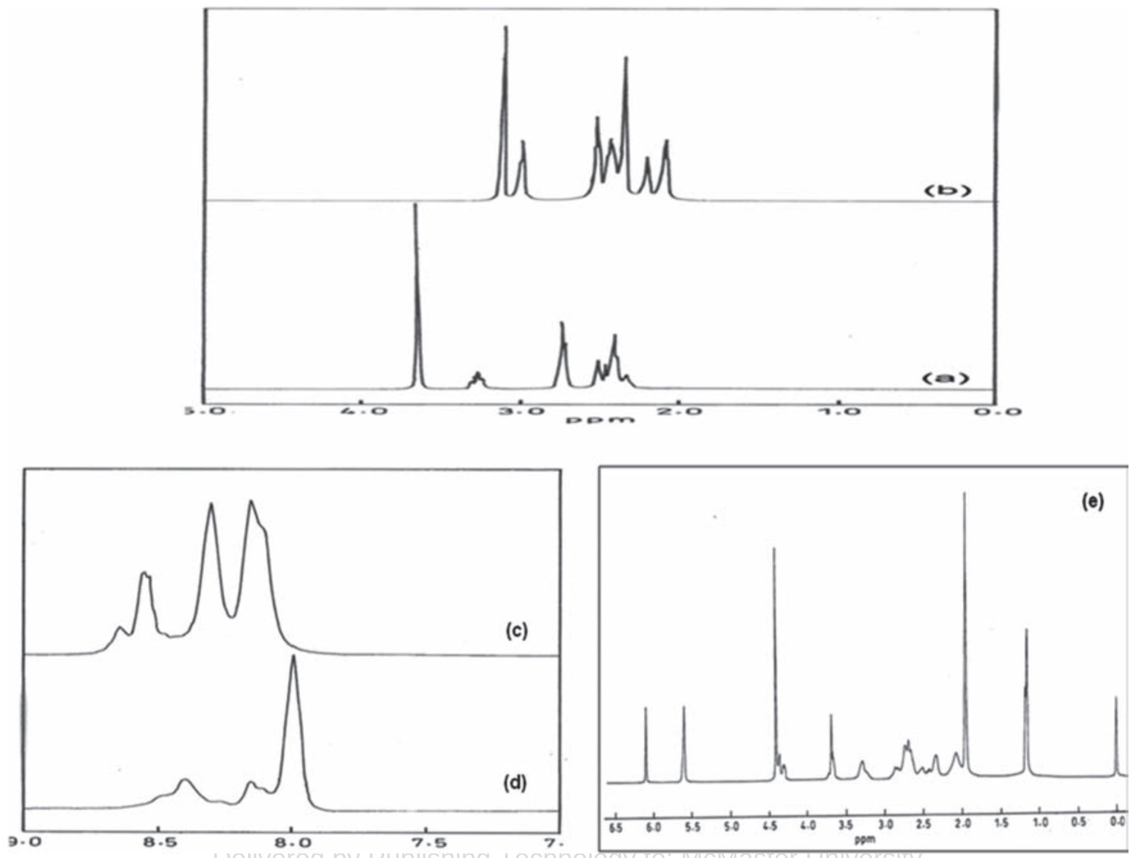


Figure 3. 1HNMR spectrum of (a) PAMAM G 0.5 (b) PAMAM G1.0; Expanded 1HNMR spectrum of (c) PAMAM G1.0 (d) PAMAM G1.0 Q HCl (e) PAMAM G1.0 EGDMA.

4.2.4. Scanning Electron Microscopy

The scanning electron micrographs of PAMAM dendritic polymer network before and after quaternization are presented in Figure 5. Polymer showed more roughness in the surface but no porosity was observed on polymer after the quaternization. The increase in roughness and irregularity of the polymer was probably due to heterogeneous reaction of acid on polymer and the structure is intact and not degraded by acid an essential feature for antimicrobial system.

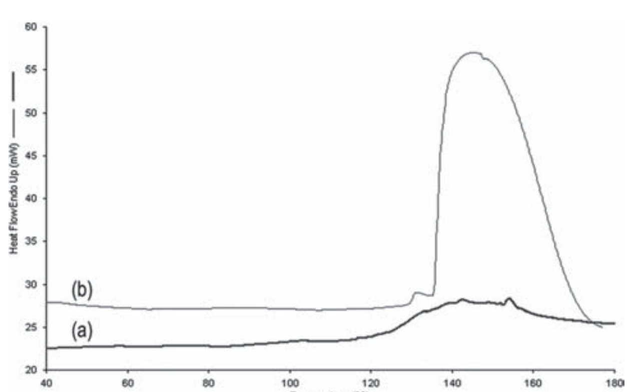


Figure 4. DSC curves of PAMAM (a) G1.0 EGDMA (b) G1.0 EGDMA Q OI.

4.2.5. Gel Content

Quaternary ammonium poly(PAMAM G1.0-EGDMA Q OI) was found to be highly insoluble in chloroform and its gel content was evaluated as 99.94% indicating highly cross linked structure of dendritic polymer network and its high stability in organic solvent.

4.2.6. Swelling Studies

The swelling behavior of the poly(PAMAM G1.0 EGDMA) and poly(PAMAM G1.0 EGDMA) Q OI in distilled water are shown in Figure 6. Quaternized dendritic polymer showed more water swelling property (19.7%) than its non-quaternized version (11%). Maximum percentage of swelling was achieved within 30 minutes after immersing in water. Increased amount of swelling can be attributed to the increase in charge density of the quaternized product. Irregular surface of quaternary polymer was found to hold more water than the smooth non-quaternized polymer. Interaction of water molecules increases with quaternization suggesting that the material will hold more water molecules as a disinfectant which is essential for their working.

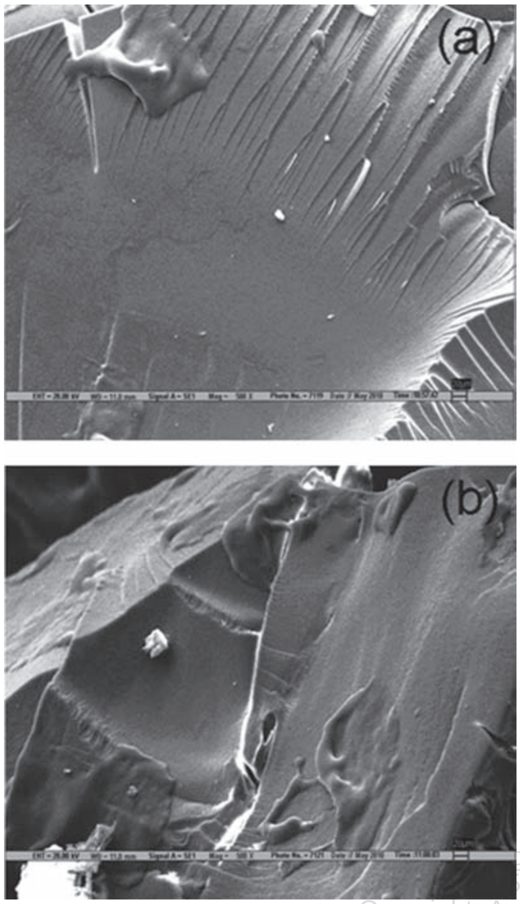


Figure 5. SEM micrographs of (a) PAMAM G1.0 EGDMA (b) PAMAM G1.0 EGDMA Q OI.

4.2.7. Leaching Studies

Reactant EDA and liquid product PAMAM G1.0 were soluble in water and showed strong absorption at 243 nm and 220 nm respectively in UV-Visible spectrophotometer. Synthesized dendritic network showed no significant absorbance in the entire UV-Visible-Partial NIR range of wavelengths from 190–1100 nm even though the sample was kept in water for 30 days. This confirmed that the synthesized solid compound was stable and no leaching of chemical components occurred from dendritic polymer in water. No weight loss was also observed from the polymer after keeping them for one month in water which is attributed to stable structure of the compound eventually indicating water stability and their potential use for disinfection purpose.

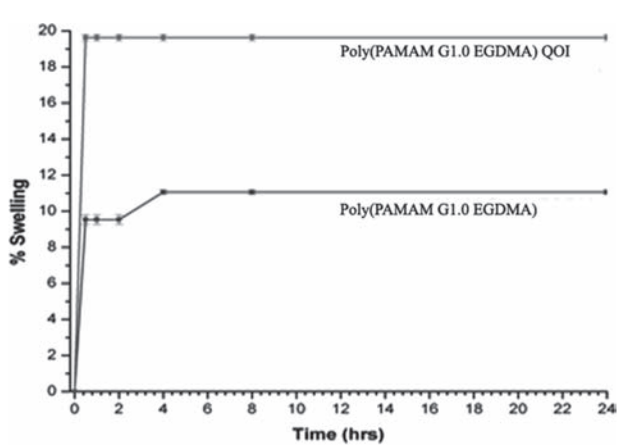


Figure 6. Swelling behavior of poly(PAMAM G1.0 EGDMA) and poly(PAMAM G1.0 EGDMA) Q OI.

4.3. Evaluation of Antimicrobial Activity of PAMAM G1.0 QHCl and Poly(PAMAM G1.0-EGDMA) QOI

Table I compiles the antimicrobial activity of quaternary ammonium PAMAM dendrimer and dendritic polymer network against *E coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis*, common bacterial contaminants present in water. The results showed that both liquid quaternary ammonium dendrimer and solid dendritic polymer network are highly effective against very high count of common Gram-negative and Gram-positive bacteria present in 10^8 cfu/mL concentration. It was observed that their antimicrobial activity increased with increase in time of incubation and concentration. 10 mg/L PAMAM G1.0 QHCl was efficient to kill 100% bacteria within a contact time of just 2 min and 10 mg/L of quaternary ammonium dendrimer showed almost double microbial killing property than 5 mg/L of compound. These results highlight the potency and efficiency of synthesized dendrimers since they are able to mitigate very high concentration of 10^8 CFU/mL and they would be effective against lower concentrations of bacterial usually contaminating residentials and hospitals. This can be attributed to the fact that polycationic QACs work on contact mechanism for killing bacteria by disrupting and permeating lipid bilayer membrane of bacterial cell. Negatively charged lipopolysaccharide and peptidoglycan layer of Gram negative bacteria as well as lipoteichoic acid and teichoid acid layer in Gram-positive bacteria facilitate interaction and eventual entry of positively charged dendrimers disrupting cellular integration. Log reduction value, denoting the change (decrease) of microorganism population relative to the starting inoculums, of quaternary ammonium PAMAM dendrimer was calculated for the concentration 5 mg/L with incubation period of 2 min (Table II). By definition, the log reduction value of 8 indicates 100% killing of 10^8 CFU/mL of bacterial concentration. Among all bacteria, highest log reduction value of 7.0 was observed with the bacteria *P. aeruginosa* whereas *B. subtillis* showed lowest log reduction value (6.38) probably as during adverse conditions these bacteria develop endospores consisting of thick cell wall which can withstand harsh conditions.[37] Dendritic polymer network, poly(PAMAM G1.0 EGDMA)

Table I. Antimicrobial evaluation of quaternary ammonium PAMAM dendrimer and dendritic polymer by colony count method.

Quaternary ammonium dendrimers	Incubation time (min.)	Bacterial survival (CFlVmL)							
		5 mg/L dendrimers				10 mg/L dendrimers			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
PAMAM G1.0 Q HCI	2	13±4	10±2	23±6	24±7	0	0	0	0
	5	8±2	9±4	12±5	16±3	0	0	0	0
	10	0	0	0	0	0	0	0	0
Poly(PAMAM G1.0 EGDMA) QOI	2	19±5	16±7	33±5	41±6	3±1	3±3	5±3	6±4
	5	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0

QOI required higher contact time of 5 min for 100% killing as antimicrobial action is through the contact of antimicrobial polymer with the cell walls of bacteria without releasing any active agents.[9] Lower log reduction values (6.38–6.79) were observed for 5 mg/L poly(PAMAM G1.0 EGDMA) QOI compared to PAMAM G1.0 Q HCl (Table II) indicating that due to insolubility of dendritic polymer in water they take more time to make contact with the microbes. These structures are new age antibacterials offering advantage of efficient contact killing based biocidal property without releasing any component like drug or biocidal loaded polymers.

4.4. Evaluation of MIC and MBC of PAMAM G1.0 Q HCl

Minimum inhibitory concentration and minimum bactericidal concentration of the synthesized quaternary ammonium PAMAM dendrimer was found to be 2.5 mg/L and 4 mg/L respectively against various tested bacteria. These values are in agreement with other reported polymeric antimicrobial formulations[38, 39] and are better than some of the iodine based antibacterial products.[40, 41]

4.5. Evaluation of Antimicrobial Efficiency Poly(PAMAM G1.0 EGDMA) QOI for Repetitive Use

It was observed that there was no growth of bacteria in any of the nutrient agar plats till twenty five cycles except in the negative control. The results demonstrate its excellent efficiency as antimicrobial agent even with very high concentration of 10^8 CFU/mL of bacteria.

Table II. Comparative log reduction value observed for PAMAM G1.0 QHCl and poly(PAMAM G1.0 EGDMA) Q OI.

Dendrimer (5 mg/mL)	Contact time = 2 min (Initial bacterial concentration = 10^s cfu/mL)			
	E. Coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
PAMAM G1.0 Q HCL	6.89	7.00	6.64	6.62
Poly(PAMAM G1.0 EGDMA) QHCl	6.21	6.79	6.48	6.38

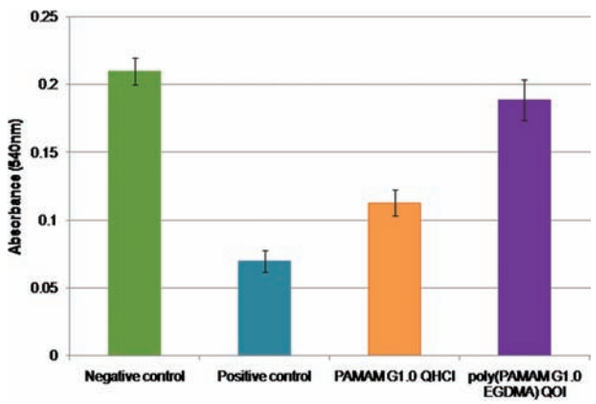


Figure 7. *In-vitro* toxicity assay (MTT assay) of synthesized quaternary ammonium dendrimer and dendritic polymer network.

4.6. *In Vitro* Toxicity Assessment of PAMAM G1.0 QHCl and Poly(PAMAM G1.0-EGDMA) QOI by MTT Assay

MTT assay results of quaternary ammonium PAMAM dendrimer and dendritic polymer network are shown in Figure 7. Higher the cytotoxicity of the tested material, the lesser will be the metabolic activity of the cells leading to lower absorbance value. The results indicated that Jurkat cells were proliferating and remained viable after 24 h of exposure to quaternary ammonium dendrimer and dendritic polymer network. PAMAM G1.0 QHCl showed absorption value of 0.11 compared to 0.21 of negative control and 0.074 value of positive control, indicating more number of surviving cells and hence its less toxic nature. Cytotoxicity of the PAMAM showed further reduction on polymerization as indicated by the higher value of absorption (0.19), indicating no leaching of active compounds in water medium.

5. CONCLUSIONS

This study reports for first time the quaternization of PAMAM with HCl to form water soluble antimicrobial PAMAM dendrimer. It also illustrated direct functionalization with EGDMA to make dendritic polymeric network and its eventual quaternization. Both quaternary ammonium dendrimer (PAMAM G1.0 QHCl) and dendritic

polymer network (poly(PAMAM G1.0-EGDMA) Q OI) showed significant antimicrobial activity against Gram-positive and Gram-negative bacteria. 5 mg/L water soluble PAMAM G1.0 QHCl was found to be efficient to disinfect all types of tested bacteria within an incubation time of just 2 minutes. Moreover, 10 mg/L of water insoluble poly(PAMAM G1.0 EGDMA) QOI also disinfected 100% bacteria in water within a contact time of 5 minutes and maintained their activity even after 25 cycles. Antimicrobial action of quaternary ammonium dendrimers was based on contact killing mechanism in which no active compounds leach out into the surrounding medium. MTT assay of the synthesized quaternary ammonium compounds revealed that they are not toxic to mammalian cells but are efficient to kill microorganisms. Application of synthesized quaternary ammonium water soluble PAMAM can be extended towards the sterilization of biomedical devices where autoclaving or harsh chemical treatment is not possible. Quaternary ammonium dendritic polymer network has the full scope of their utilization in designing drinking water disinfection cartridge.

ABBREVIATIONS

QAC	Quaternary ammonium compounds
G	Generation
Q	Quarternized
PAMAM	Polyamido amine
OI	Octyl iodide
PAMAM-EGDMA	Polyamido amine-ethylene glycol dimethacrylate.

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