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Self-Assembled Polyamidoamine Dendrimer on Poly (methyl methacrylate) for Plasmonic Fiber Optic Sensors

M. Divagar[+],[a] Jain Saumey[+],[b, e] Jitendra Satija,*[c] and V. V. R. Sai*[d]

Abstract: We report a novel one-step polyamidoamine (PAMAM) dendrimer based polymethyl methacrylate (PMMA) surface functionalization strategy for the development of polymeric optical fiber (POF) based plasmonic sensors utilizing gold nanoparticles (AuNP). Simple contact angle measurements over PMMA sheets reveal the ability of the dendrimers to strongly bind to PMMA surface without additional acid/alkali pretreatment, unlike the conventional hexamethylene diamine (HMDA) based surface modification. Subsequently, U-bent POF probes with high evanescent wave absorbance sensitivity were exploited for relative quantification of the surface amine groups using fluorescein isothiocyanate (FITC) binding and efficient chemisorption of gold nanoparticles (AuNP) in order to identify the optimum conditions viz. dendrimer concentration, incubation time and dendrimer generation. While FITC binding showed a proportional increase in amine functional density with PAMAM concentration and time, interestingly the AuNP (40 nm) binding studies revealed the formation of loose PAMAM multilayers and their desorption. PAMAM (G4) concentration as low as 5 mM and incubation time of 24 h provide faster binding rate with densely packed AuNP and the RI sensitivity of ~15 ($A_{546\,nm}$/RIU). This simpler and inexpensive strategy could also be exploited for the development of functional PMMA substrates for various applications including nanotechnology, bio-imaging, drug delivery and analytical separations.

1. Introduction

Polymeric optical fibers (POF), especially POF made of polymethylmethacrylate (PMMA), are widely used for communications and physical sensing applications owing to their high transparency in visible and near-infrared (NIR) regions.[1] These fibers have also been explored in developing highly sensitive fiber optic chemical and biosensors by exploiting evanescent wave absorption (EWA),[2] phase modulation[3] or refractive index (RI) loss phenomena.[4,5] POF offer distinct advantages over silica fiber including low-cost (multi-mode fibers in particular), ease in machinability and ruggedness in addition to comparable performance. Recently, POF has been exploited in development of plasmonic fiber optic sensors (P-FOS) based on surface plasmon resonance (SPR) and localized SPR (LSPR).[6–9] Noble metal nanostructures were deposited on a POF surface by means of either sputter deposition or chemisorption of nanoparticles from solution phase.[6,7,10] Sputter deposition on PMMA based POF is associated with formation of craters due to evaporation of water content,[10] leading to surface deformation and inconsistency in the end result. On the other hand, the chemisorption of nanoparticles requires PMMA surface modification by multi-step treatment processes to generate active functional groups on its surface[11–14] as discussed below.

PMMA, from a physico-chemical perspective, is a transparent thermoplastic that is widely used as a substitute for inorganic glass substrates due to its mechanical strength, shatter-resistant nature, low-weight and machinability.[13] Despite a number of advantages, their chemical inertness is one of the major limitations in utilizing PMMA as a functional substrate for various biomedical applications. Till date, several chemical, physical or physicochemical methods have been investigated for PMMA surface modification in order to introduce functional groups such as amine, thiol, carbonyl and carboxylic groups.[15–20] Most of these methods involve the pretreatment of PMMA surface with strong acids or alkali followed by conjugation of bifunctional agents. Several amine based homo bi-functional linkers including N-lithioethylene diamine,[12] ethylene diamine,[21] hexamethylene diamine (HMDA)[11] and 1,3-diaminopropane[18] or amine based polymeric agents such as polyethyleneimine (PEI)[22] and diamino polyethylene glycol (PEG)[23] have been reported to functionalize the PMMA substrates.

As an alternative to the aforementioned chemical methods that involve strong acid/alkali pre-treatment, several physical methods have been reported that are relatively environmental friendly and less tedious. For example, Long et al. (2006),[24] suggested he use of water-vapor plasma functionalization

[a] M. Divagar[+]
Interdisciplinary Research Program
Department of Biotechnology and Department of Applied Mechanics
IIT Madras, Tamil Nadu, 600036

[b] J. Saumey[+]
School of Biosciences and Technology, VIT Vellore, Tamil Nadu, 632014

[c] Dr. J. Satija
Centre for Nanobiotechnology (CNBT) VIT Vellore, Tamil Nadu, 632014
E-mail: jsatija11@gmail.com

[d] Dr. V. V. R. Sai
Department of Applied Mechanics, IIT Madras, Tamil Nadu, 600036
E-mail: vvrsai@iitm.ac.in

[e] J. Saumey[+]
KTH Royal Institute of Technology, School of Electrical
Engineering and Computer Science, SE-16440 Kista, Sweden

[+] These authors contributed equally.

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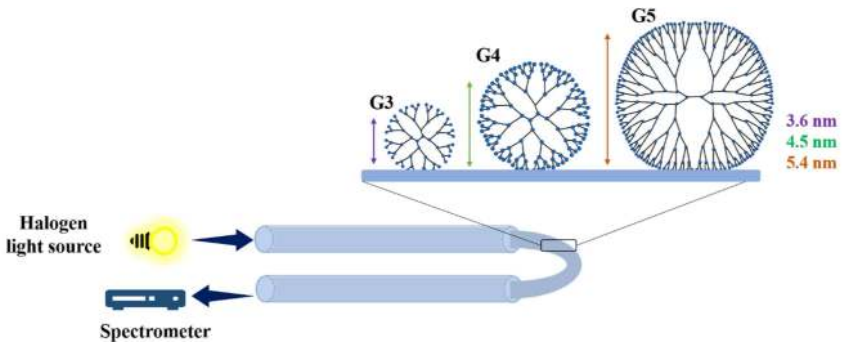

Figure 1. Schematic showing the experimental set-up with dendrimer-coated PMMA-POF coupled to a halogen light source and fiber optic spectrometer. Inset: PMMA surface modification with PAMAM dendrimer of different generations (G3, G4 and G5) and their dimensions explored in this study.

followed by attachment of organosilanes on PMMA surface to obtain amine functional groups. In a different study, UV irradiation has been reported to generate carbonyl groups on a PMMA-POF surface by Nugen et al., (2009).[21] Recently, our group has demonstrated the argon plasma treatment-based functionalization of POF.[25] Although many of these physical and chemical methods are efficient enough to functionalize the PMMA surface, most of these suffer from poor chemical reactivity, low surface energy, presence of weak cohesive layer and large batch to batch variations.[18] Thus, development of a facile and robust surface modification strategy for PMMA substrate is still a challenge.[26]

Over the past few decades, dendrimers have been widely explored for surface modifications of various technologically important substrates including silicon, glass, mica, gold, quartz and silica.[27] Dendrimers are three-dimensional, hyperbranched, and monodispersed nanoscale polymeric macromolecules with a very high surface functional group density.[28] These supramolecules have garnered much interest for designing sensor matrix because of the unique combination of their dendritic properties and capability to generate three-dimensional, highly dense functional sensor matrix which can help in amplification of the sensor signal. In addition, the ease in dendrimer synthesis chemistry and conjugation chemistry provide flexibility to choose dendrimer with homo or hetero multifunctional groups to generate the desired functionalities at sensor surface.[27,29,30] For example, amine-terminated PAMAM dendrimers have been covalently bound to 11-mercaptoundecanoic acid (11-MUA) functionalized gold surfaces by means of carbodiimide coupling chemistry for biosensing applications.[31] In another approach, amine-terminated dendrimer films have been formed on silica substrates containing hydroxyl groups by means of amide bond formation using 1,1′-carbonyldiimidazole linker.[32–34]

In this work, we report a novel one-step strategy for PMMA surface modification with amine-terminated PAMAM dendrimer to achieve dense and active amine functional groups on POF surface to realize P-FOS for chemical and biosensing applications. The optimal conditions for the dendrimer binding to PMMA surface with respect to acid/alkali pre-treatment were determined by means of the contact angle measurement on PMMA sheets. Further, the high EWA sensitivity of the U-bent POF probes was exploited to evaluate the optimum dendrimer concentration, incubation time and generation to obtain a highly dense surface functional groups by using fluorescein isothiocyanate (FITC) binding to amine groups. These conditions were verified by carrying out detailed investigations for efficient chemisorption of AuNP to the PAMAM dendrimer coated PMMA POF fiber probes. These studies reveal several interesting aspects of dendrimer interaction with PMMA surface such as multilayers for high concentrations and different AuNP binding rates for various combinations of dendrimer concentration and incubation time as well as dendrimer generation.

2. Results and Discussion

2.1. PAMAM Dendrimer Surface Modification

2.1.1. Characterization of Binding of G4 PAMAM Dendrimer to PMMA Surface

To establish a proof-of-concept for the PAMAM dendrimer based PMMA surface modification, initial studies were carried out using PMMA sheets and G4 PAMAM dendrimers with concentration and incubation time of 5 μM and 24 h respectively. The changes in the water contact angle due to the acid/alkali pre-treatments as well as PAMAM binding were investigated to determine the role of pre-treatment in PAMAM based PMMA surface functionalization (Table 1).

Table 1. Water contact angle measurements obtained from PMMA sheets subjected to acid/alkali pre-treatment followed by G4 PAMAM dendrimer binding (n = 3).

Pre-treatment	Water contact angle	
	After pre-treatment	After PAMAM (G4) binding
Pristine (control)	112.14° ± 7.90	61.38° ± 7.71
1 M H_2SO_4	97.17° ± 1.11	81.62° ± 6.22
MeOH:HCl	93.33° ± 5.50	77.30° ± 6.10
MeOH:HCl + 1 M H_2SO_4	83.66° ± 7.76	65.11° ± 3.11

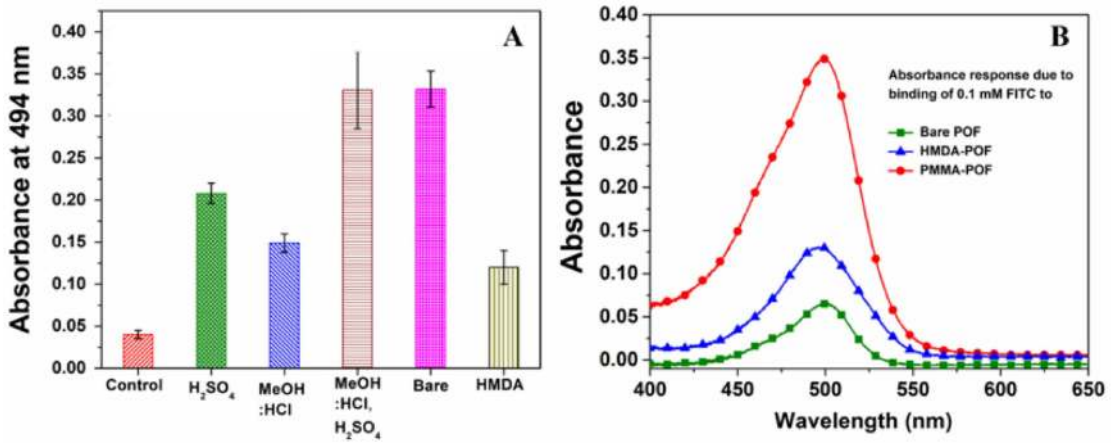

Figure 2. (A) Absorbance response due to FITC binding to (i) control (without G4 PAMAM dendrimer surface modification) and G4 PAMAM dendrimer-coated U-bent POF probes that are pre-treated with (ii) 1 M H2SO4, (iii) MeOH-HCl, (iv) their combination and (v) without any pre-treatment. (B) Absorbance response from U-bent POF probes due to FITC binding to bare (spheres), HMDA functionalized following an acid pre-treatment (triangles) and G4 PAMAM functionalized (squares) probes U-bent PMMA-POF.

A considerable drop in the contact angle, from 112° to around 90°, was observed upon acid/alkali pre-treatment, of which MeOH:HCl followed by 1 M H_2SO_4 gave rise to a significant change of ~30°. This indicates the ability of the pre-treatment to improve the hydrophilicity of the surface by generation of additional hydroxyl groups. We attribute it to hydrolysis of ester groups present on the PMMA surface.[19] More importantly, G4 PAMAM dendrimer treatment leads to a further decrease in the water contact angle for pristine and pre-treated surfaces. In comparison to pre-treated PMMA surface, a significant reduction in the water contact angle up to ~50° was observed for pristine PMMA sheets directly modified with G4 PAMAM dendrimer without any pre-treatment. Hence, we anticipate a significant binding of PAMAM dendrimer to PMMA surface with a negligible influence of acid/alkali pretreatment process on PAMAM binding. Here, the potential interaction of amine groups on PAMAM dendrimers with the residual hydroxyl groups on PMMA surface before pretreatment itself and subsequent formation of hydrogen bonds and/or electrostatic interaction could be attributed as a possible reason for these observations.[35,36] The interaction of PAMAM dendrimer to bare PMMA surface was sufficiently strong enough to withstand repeated washing steps with DI water. These results suggest that PAMAM dendrimers binds firmly to the PMMA surface.

2.2. Relative Estimation of Amine Functional Groups using U-bent POF Probes

Several parameters including PMMA surface pre-treatment, PAMAM concentration, incubation time and generation of dendrimer are known to play a critical role in the efficiency of the surface modification process. Highly sensitive U-bent POF probes can be exploited as simpler tool for relative estimation of functional groups and thus investigate the influence of the above process parameters on the efficiency of surface modification.

2.2.1. Influence of PMMA Surface Pretreatment

The U-bent POF probes were functionalized with G4 PAMAM dendrimer (5 μM, 24 h) following the above acid/alkali pretreatment protocols. The active amine functional group density on these probes was compared by measuring the EWA response upon binding of FITC molecules. Amongst the pre-treatments considered, the combination of MeOH:HCl and 1 M H_2SO_4 showed a significant increase (seven-fold) in EWA response in comparison to the bare U-bent POF probe (indicated as control) (Figure S2A, S2B). However, G4 PAMAM dendrimer bound PMMA-POF probes without any pre-treatment (indicated as bare) also showed a similar response. These results are in agreement with the trend observed for contact angle measurements and show that the efficient dendrimer binding can be achieved even without any pre-treatment. The experiments were repeated thrice and the EWA response due to FITC binding was quite repeatable and stable with water wash, showing the robustness of PAMAM dendrimer binding to the PMMA surface.

Further, comparison of amine functional group density obtained by PAMAM dendrimer based surface modification strategy with that of homo bifunctional agent such as HMDA reveal at least 2 fold higher absorbance response due to FITC binding (Figure 2A, 2B). This could be attributed to the large globular nature of the dendrimer with relatively large functional groups available for binding in comparison to the linear chain-like molecules ch as HMDA.

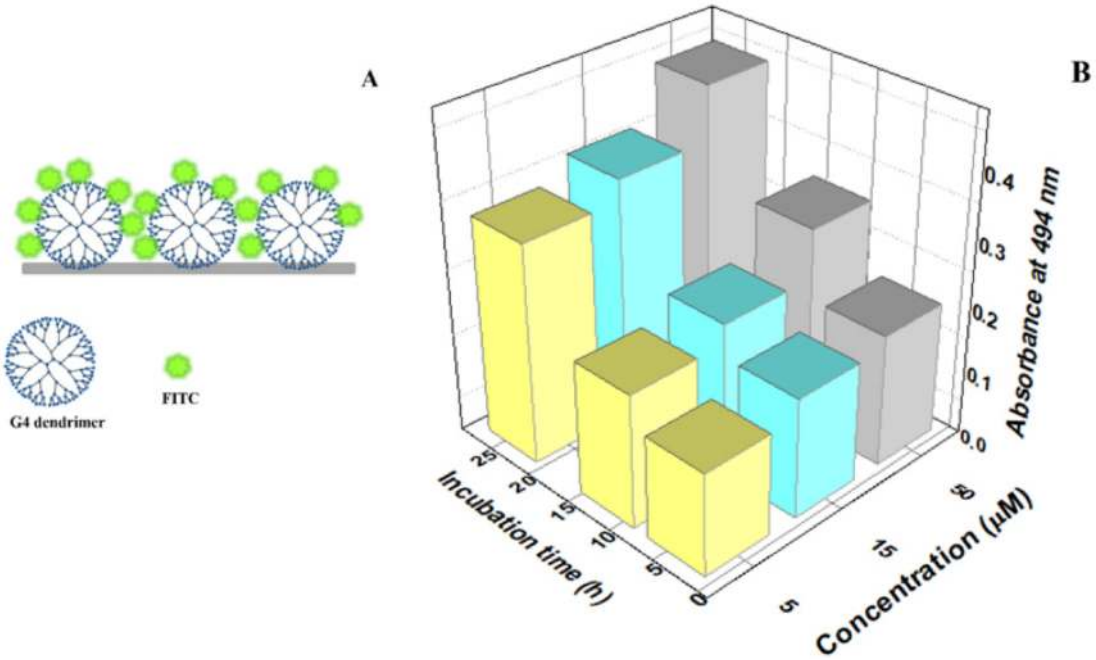

Figure 3. (A) Schematic representation of FITC binding to G4 PAMAM dendrimer on U-bent PMMA-POF probe surface and (B) saturated absorbance response at 494 nm of U-bent POF with varying incubation time and concentration of dendrimer solution (the probes were incubated in FITC solution of 0.1 mM concentration).

2.2.2. Influence of Incubation Time and Concentration

In order to establish an optimal protocol for dendrimer immobilization, the decladded fiber probes were incubated in G4 PAMAM dendrimer solution of varying concentrations (5, 15 and 50 μM) each for 4 h, 12 h and 24 h. The influence of different combination of dendrimer concentration and incubation time was evaluated by using EWA response due to FITC binding. The real-time absorbance response (at 494 nm) of these probes due to FITC binding is shown in Figure S3A, S3B and S3 C. Figure 3 shows the absorbance values obtained for all the nine probes coated for different time and concentration of dendrimer as a 3D matrix plot. These results show an increase in FITC binding and hence the number of available amine groups as a function of both dendrimer concentration and incubation time. The incubation time of 24 h consistently gave rise to an increased dendrimer immobilization in comparison to 4 and 12 h of incubation time. An increase in dendrimer concentration from 5 to 50 μM also resulted in a considerable improvement in the number of amine groups.

2.3. Development of Plasmonic POF Sensors

The development of plasmonic POF probes involves chemisorption of AuNP to amine functional groups on the PAMAM treated PMMA surface with bond energies in the range of 5–10 kcal/mol.[37] The extent of AuNP binding depends on the availability of amine groups accessible at the fiber surface. Hence, experiments were carried out to investigate the optimum dendrimer concentration and incubation time for a suitable PAMAM dendrimer surface coverage for efficient AuNP binding. Two interesting observations were made during these experiments.

Firstly, when the U-bent probes treated with 50 μM PAMAM were incubated in AuNP solution, an agglomeration of AuNP suspension (colour change from pink to purple) within a few minutes upon exposure to the probes was observed (Figure 4B), as opposed to that of 5 and 15 μM concentrations. However, when these probes were washed and again incubated in fresh AuNP suspension, significant EWA response was obtained without any agglomeration, just similar to the probes treated with lower concentrations of PAMAM. We anticipate formation of dendrimer multilayers on the U-bent POF probes surface for this unanticipated observation. Higher dendrimer concentration is known to result in the formation of multiple layers of dendrimer on a sensor surface with the help of electrostatic interactions and hydrogen bonding.[27,35,38] While adhesion of the additional dendrimers to the primary monolayer appears to withstand several washing steps with DI water, chemisorption of AuNP of high molecular weight to these dendrimers seems to exert sufficiently large force to cause desorption of these additional dendrimers from the surface. We anticipate the desorption of dendrimer coated AuNP and crosslinking with the monodispersed AuNP in the solution phase as the primary reason for agglomeration of AuNP colloids observed in case of U-bent probes treated with 50 μM PAMAM. To obtain a deeper insight, the number of dendrimer molecules necessary for a saturated surface coverage was calculated. Theoretical estimations show that 30 μL of 5 μM G4 PAMAM aqueous solution contains ~9×10^{13} dendrimer molecules, while 2.46×10^{11} mole-

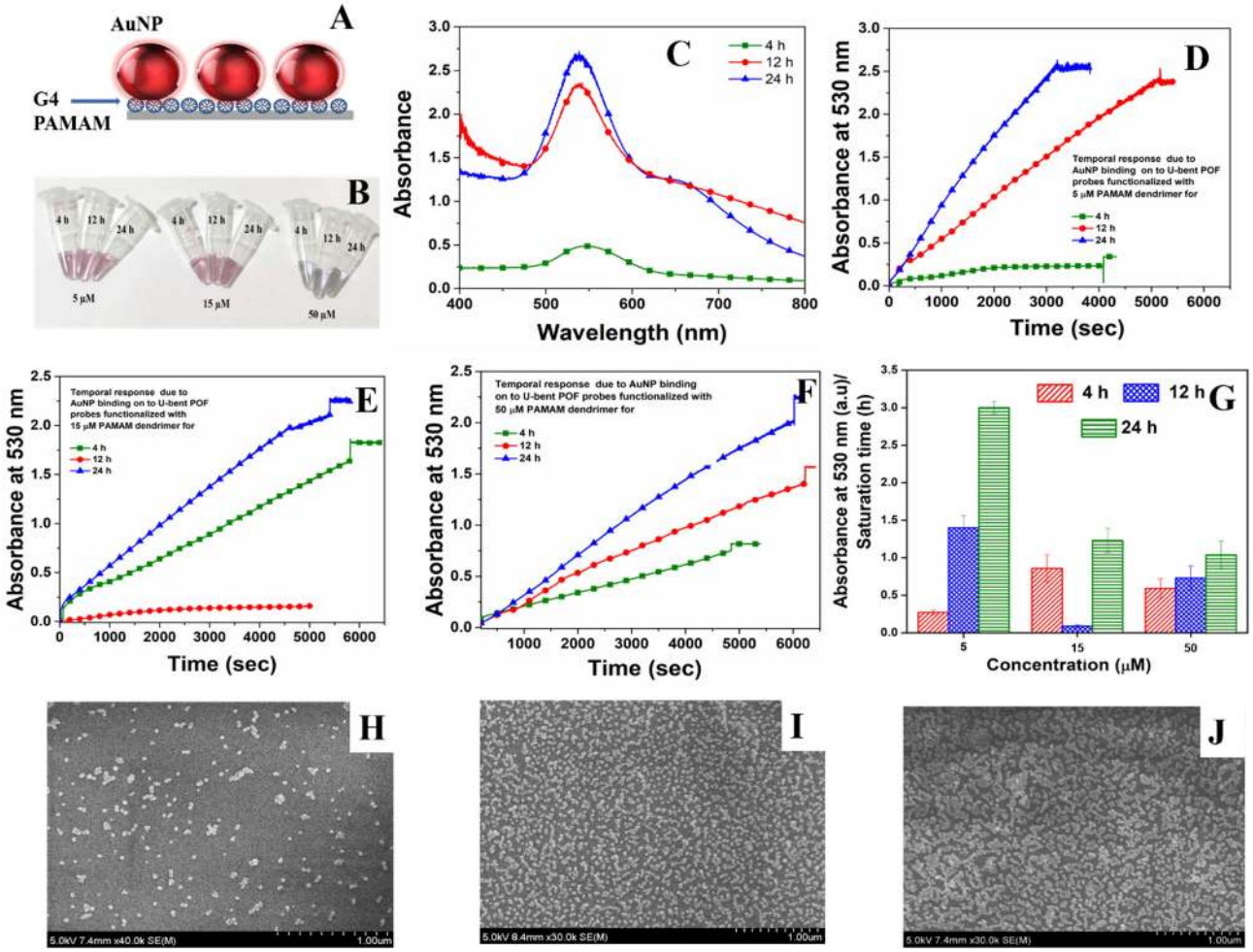

Figure 4. (A) Schematic representation of AuNP binding to G4 PAMAM dendrimer on U-bent POF probe surface. (B) Photographic image of freely suspended (5 and 15 μM) and aggregated (50 μM) AuNP colloidal solutions incubated with PAMAM modified U-bent probes subjected to varying dendrimer concentration and incubation time. (C) Absorbance response due to binding of AuNP obtained from U-bent POF probe functionalized with 5 μM G4 PAMAM dendrimer concentration and 4, 12, 24 h of incubation time. (D, E, F) Temporal response at 530 nm from PAMAM modified U-bent POF probes subjected to 5 μM, 15 μM and 50 μM concentrations and incubated for 4 h, 12 h and 24 h. (G) AuNP binding rate to U-bent POF probes functionalized with G4 PAMAM dendrimer at varying incubation time using varying dendrimer concentration. The binding rate was calculated by taking the ratio of saturated EWA response at 530 nm and time taken to reach the saturation response (h). (H, I, J) SEM images of AuNP bound U-bent PMMA-POF surface coated with 5 μM G4 PAMAM dendrimer by varying the incubation time such as 4 h, 12 h and 24 h.

cules are sufficient to obtain a saturated surface coverage over a 1 cm long U-bent fiber probe, assuming a closely packed dendrimer layer. Thus, clearly indicates a high probability of obtaining dendrimer multilayers on U-bent POF surface for higher concentrations such as 50 μM G4 PAMAM.

Secondly, a high EWA response due to AuNP binding for all the treatments except for (5 μM, 4 h) and (15 μM, 12 h), however at varying binding rates as shown in Figure 4D–4F. Consistently, 15 μM, 12 h showed a very low EWA response in comparison to other treatments. We anticipate formation of loosely bound dendrimer monolayer with 15 μM, 12 h, similar to 50 μM concentration, that are vulnerable to detach from the fiber surface upon AuNP binding. However, 15 μM, 24 h gave a saturated EWA response, which could be due to the longer incubation time promoting strong adsorption of dendrimer molecules to the fiber probe surface. The absorbance spectral response due to the saturated surface coverage of AuNP on U-bent probes treated with 5 μM for 4, 12, 24 h incubation times was as shown in Figure 4C. In comparison to the spectral characteristics of AuNP colloids (Figure S1), a plateau around 650 nm was observed, indicating the aggregation of a considerable fraction of the AuNP bound to the PAMAM-modified surface as shown in their respective SEM images in Figure 4G–4I. An important observation was that the AuNP binding kinetics obtained from the EWA responses were significantly different for the various treatments and consistent over 3 different sets of experiments (Figure 4G) was considered to identify the optimum process. A consistently high AuNP binding rate was observed for the U-bent POF probes treated with (5 μM, 24 h) followed by (5 μM, 12 h). Thus, taking these two fibers for further validation, it found that though both the fiber probes (5 μM, 12 h and 5 μM, 24 h) reached a maximum

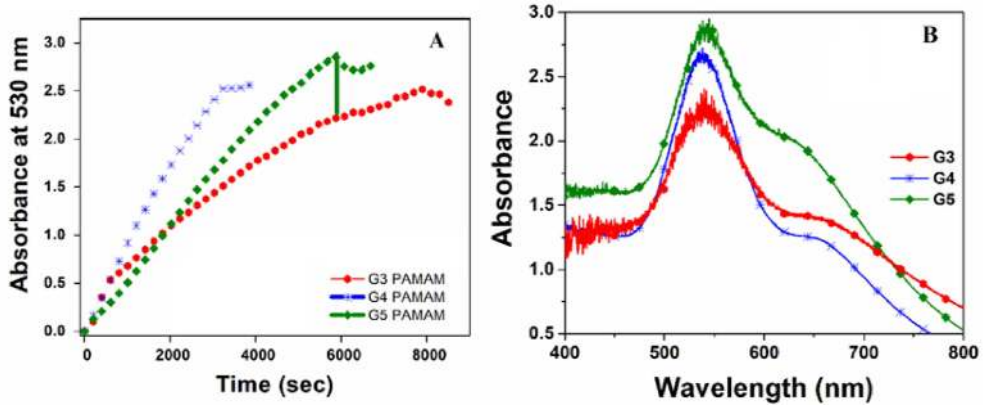

Figure 5. (A) Temporal response at 530 nm and (B) absorbance response obtained from U-bent POF probes functionalized with dendrimer of a different generation and thereafter, subjected to AuNP (OD 1.5).

absorbance (~2.5 absorbance value at 530 nm), the distribution of AuNP significantly varies as evident in the SEM images. The absorbance spectrum also shows the presence of significant plasmon resonance coupling band for 5 μM, 24 h in comparison to 5 μM, 12 h (Figure 4C).

Overall, these results suggest depending the AuNP binding kinetics G4 PAMAM dendrimer of 5 μM concentration and 24 h of incubation is optimum to obtain an LSPR surface with dense packing of AuNP and the packing density could be varied by reducing the incubation time and using AuNP colloidal solution of lesser OD.[39]

2.3.1. Influence of Dendrimer Generation

An interesting aspect of dendrimers is their availability in various sizes (generation) with a desired number of terminal functional groups. Hence, the use of higher generation dendrimer is anticipated to enhance the active functional group density on a sensor matrix. Here, the influence of dendrimer generation on surface modification of U-bent POF probes was investigated by subjecting them to G3, G4 and G5 PAMAM dendrimers (5 μM, 24 h). A dendrimer concentration and incubation time of 5 μM and 24 h respectively was chosen as it has faster AuNP binding kinetics and resulted in high density of AuNP on the surface. Initial studies with FITC show an increase in the EWA response with the PAMAM dendrimer generation, indicating an improvement in the availability of the total number of active terminal functional groups (Figure S4A, S4B). In comparison to G3 PAMAM, the improvement in surface functional density was marginal for G4 dendrimer, whereas a significant increase was observed with G5 dendrimer. This could be attributed to the cumulative effect of the dendrimer surface coverage over the sensor and the number of functional groups over each dendrimer. It is important to note that the dendrimer surface coverage is influenced by its surface charge. For example, the G5 dendrimer has ~four times higher functional groups and hence proportionally higher surface charge in comparison to G3, resulting in larger repulsive forces among the dendrimers. Thus, the surface number density of G5 is anticipated to be significantly lower than that of G3 dendrimer assembly, which is also evident in AFM images (Figure S5).[40]

In case of AuNP binding, EWA response due to a saturated AuNP surface coverage (~2.5 absorbance at 530 nm) was observed from U-bent POF probes treated with G3, G4 and G5 dendrimer (Figure 5). The AuNP binding kinetics on fiber probes were significantly different. The results show a consistently faster AuNP binding rate for G4 PAMAM dendrimer modified probes followed by G5 and G3 (saturation response reached after 3000, 4800 and 8000 s respectively) as shown in Figure 5A. The highest binding rate observed with G4 could be attributed to its optimum density and uniform coverage by the dendrimer on the sensor substrate which is evident from the AFM images (Figure S5). In addition, the respective absorbance spectral responses are shown in Figure 5B, where a plateau at 650 nm for coupled plasmon resonance band due to a considerable AuNP aggregation was observed more pronouncedly for G3 and G5 dendrimer treatments.

These results showed that an efficient P-FOS using AuNP of 40 nm diameter could be obtained using G4 PAMAM dendrimer with concentration and incubation time of 5 μM and 24 h, respectively. Also, the possibility of varying the AuNP surface distribution by varying the incubation time. These results demonstrate the feasibility of exploiting G4 PAMAM dendrimer to generate active and dense functional groups on POF surface for the fabrication of P-FOS. From the experimental studies, we observe that the plasmonic coupling is inevitable with the use of dendrimer molecules due to its globular structure with dense amine functional groups as opposed to homo bifunctional molecules such as HMDA. To observe the influence of coupled plasmon resonance the same optical set-up was leveraged to obtain the RI sensitivity of the P-FOS developed using the optimized surface modification conditions. The P-FOS were subjected to sucrose solutions of varying RI (1.3333 to 1.348, $\Delta RI = 0.003$) and the corresponding absorbance response was monitored (Figure 6A) and utilized to calculate the RI sensitivity. The RI studies showed a significant improvement in the RI sensitivity ($\Delta A_{546\ nm}$/RIU) of the developed P-FOS (Figure 6B),

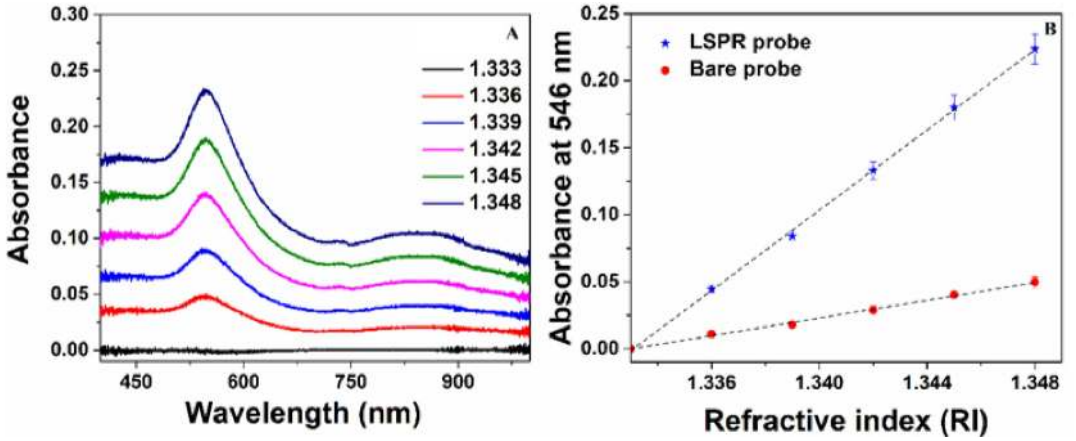

Figure 6. (A) Absorbance response and (B) RI sensitivity obtained from developed P-FOS subjected to sucrose solution of varying RI taking DI water as a reference.

where it is at least 5 times higher than that of the bare fiber probe, while it is similar to that of HMDA based surface modification previously reported by our group.

3. Conclusion

A simple, efficient and one-step strategy for PMMA surface functionalization through self-assembled amine-terminated PAMAM dendrimer is established and its application for development of POF based P-FOS is demonstrated. These studies reveal interesting outcomes: (i) High number of terminal amine groups of PAMAM dendrimer facilitates a strong and stable binding of PAMAM dendrimer on to POF without the need of any pre-activation of the surface using acid/alkali. (ii) EWA response from U-bent POF probes due to binding of FITC efficiently evaluated various aspects of surface functionalization including relative assessment of total number of functional groups. FITC binding studies suggest the ability to tune the density and distribution of surface amine functional groups by increasing the dendrimer concentration and incubation time in addition to dendrimer generation. However, AuNP binding studies clearly reveal the existence of loosely-bound multilayers of PAMAM and their desorption for higher concentrations (50 μM) as witnessed by agglomeration of AuNP. (iii) Efficient PMMA surface modification at a relatively lower concentration of 5 μM unlike homo bifunctional agents such as HMDA. In comparison to linear bifunctional cross linking agents such as HMDA, PAMAM dendrimer showed at least a 2-fold improvement in the amine functional density was obtained. Thus, suggesting that this surface modification strategy may also be utilized for immobilization of biomacromolecules such as antibodies. However, the optimum conditions need to be verified and shelf-life of surface functionalization need to be evaluated for biosensing applications.

Experimental Section

Materials

Super ESKA™ polymeric optical fibers of 500 μm diameter (SK 20, $n_{core} = 1.49$ and $n_{clad} = 1.41$) were procured from Mitsubishi Rayon Co., Ltd., Japan. PMMA sheets of 12×12 cm^2 and 3 mm thickness were procured from local market. All the chemicals used including gold chloride ($HAuCl_4 \cdot 3H_2O$, Sigma Aldrich), trisodium citrate dihydrate (Merck), amine-terminated third, fourth and fifth generation (G) PAMAM dendrimers (Sigma Aldrich), fluorescein isothiocyanate (FITC) on celite (SRL chemicals), ethyl acetate (Merck), and hexamethylene diamine (HMDA, Alfa Aesar) were of analytical grade. Deionized (DI) water obtained from Millipore was used for all experiments. The real-time absorbance spectra were recorded by coupling the U-bent optical fiber probe to a halogen light source (HL 2000, Ocean Optics Inc., USA) and a fiber optic spectrometer (USB 4000 XR1ES, Ocean Optics Inc., USA) with the help of SubMiniature A (SMA)-905 connectors and bare fiber terminator (BFT, ThorlabsInc., USA).

Synthesis of Gold Nanoparticles

The gold nanoparticles (AuNP) were synthesized by citrate-mediated reduction of $HAuCl_4$ as reported by Turkevich in 1951. Briefly, 1 mL of 12.7 mM gold chloride salt was added to 38 mL of DI water. Following, an aqueous solution of trisodium citrate dihydrate (0.349 mM, 1 mL) was added to boiling solution of gold chloride salt, while citrate to gold molar ratio was maintained 1.1. The heating was continued until the solution color turns pale pink indicating the formation of AuNP. Thereafter, the solution was allowed to cool to room temperature (RT) and stored at 4 °C. The colloidal solution of AuNP was characterized using UV-vis spectroscopy for its optical characteristics and transmission electron microscopy (TEM) for their size and shape distribution. UV-vis spectroscopic analysis shows a strong absorption peak at 530 nm. TEM analysis reveals the presence of spherical AuNP of average size of 40 nm (Figure S1).

Fabrication of U-bent POF Probes

The U-bent POF probes were fabricated as described earlier by our group.[6] Briefly, the POF of 25 cm length were made into U-shape

by inserting into a glass capillary tube of 1.9 mm inner diameter and followed by heating at 100°C for 10 min to obtain probe of bend diameter 1.4 mm. Thereafter, the bent portion of length 0.5 cm was decladded by dipping the probes in ethyl acetate over a duration of 2 mins to chemically etch the fluorinated PMMA clad. Then, the probes were washed with DI water and stored at room temperature (RT) until further use. Probe to probe variations were kept to a minimum by selecting the probes with a similar refractive index (RI) sensitivity, ~2 a.u ($\Delta A_{530\,nm}/\Delta RIU$). The RI sensitivity was obtained as a ratio of increase in absorbance at 530 nm for an increase in the RI from DI water (RI = 1.33) to sucrose solution (RI = 1.36).

Dendrimer Immobilization on U-bent POF Probes

Prior to dendrimer immobilization, the decladded U-bent POF probes, as well as clean PMMA sheets were subjected to three different types of chemical pretreatments; (i) H_2SO_4 (1 M) for 1 h at RT or (ii) MeOH:HCl mixture (1:1 v/v) for 1 h at RT or (iii) MeOH:HCl mixture (1:1 v/v) for 1 h at RT followed by dipping in H_2SO_4 (1 M) for 1 h at RT. Afterwards, the chemically pretreated POF probes and PMMA sheets were thoroughly washed with DI water and subsequently incubated in aqueous solution of PAMAM dendrimer (G4, 5 μM) for 24 h at RT. Thereafter, the probes were washed with DI water and stored at 4 °C until further use.

In order to investigate the influence of dendrimer concentration and incubation time, the decladded probes were dipped in aqueous dendrimer solution of varying concentration (5, 15 and 50 μM) over varying incubation time of 4, 12 and 24 h. Similarly, the effect of dendrimer generation was studied using PAMAM dendrimer of generations 3, 4, and 5 having 32, 64 and 128 amine terminal functional groups. For all the studies, a decladded U-bent POF probe/plain PMMA sheet without dendrimer functionalization was used as a control.

PMMA Surface Characterization

The PMMA sheets were utilized to carry out surface characterization by contact angle measurement. The amine functional group density on PAMAM dendrimer-coated POF was evaluated by optical absorbance studies using two different binding moieties, i.e. FITC molecules and AuNP. The optical absorbance measurements were carried out with the help of a halogen light source and fiber optic spectrometer as shown in Figure 1. The U-bent POF probes were coupled to the halogen light source and fiber optic spectrometer at either of its ends by using SMA connectors and BFT.

Contact Angle Measurement

The water contact angle of pristine (control) and G4 PAMAM dendrimer modified PMMA sheets with/without chemical pretreatment was measured using GBX Digidrop contact angle measurement system. The contact angle was measured with the help of 1 μL of DI water. The reported contact angle values were an average of twenty measurements repeated thrice at different points over the pristine and G4 PAMAM dendrimer modified surface.

Optical Absorbance Measurements

Dendrimer-coated U-bent POF were subjected to 30 μL of FITC solution (0.1 mM, prepared in 100 mM borate buffer, pH 8.3) or AuNP solution (OD 1.5 at 530 nm) to allow their binding to amine terminated G4 PAMAM dendrimer molecules. Absorbance spectrum of the light passing through U-bent POF probes was recorded at 494 nm and 530 nm for FITC and AuNP respectively with an integration time of 5–6 ms and averaging over 100 scans in real time until saturation of absorbance signal. An absorbance response between ~2 to 2.5 a.u is considered to be a saturated response given the limited dynamic range of the fiber optic spectrometer. Probes were washed after observing a saturation in the absorbance response. U-bent POF probes without G4 PAMAM dendrimer functionalization were used as a control.

Fluorescence Image Analysis

FITC-bound G4 PAMAM functionalized U-bent PMMA-POF probes were washed with borate buffer and observed under inverted fluorescence microscope with 10× magnification at excitation wavelength of 470–490 nm to capture fluorescence images.

AFM Image Analysis

PMMA-POF coated with PAMAM dendrimers of varying generation were characterized with non-contact mode AFM with scan rate of 1 Hz over an area of 500×500 nm^2. The distribution of PAMAM dendrimers and roughness of the surface were studied by analyzing the obtained AFM images.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: polymethyl methacrylate (PMMA) · polymeric optical fibers (POF) · dendrimers · plasmonic sensors · gold nanoparticles (AuNP) · evanescent wave absorbance

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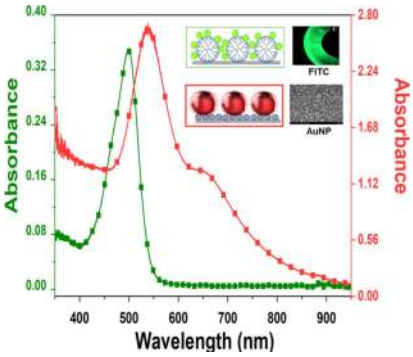
FULL PAPER

Plasmonic PMMA surfaces: A novel one-step PMMA surface modification strategy using G4 PAMAM dendrimer is established and optimized by efficiently exploiting the evanescent wave absorbance response of polymeric optical fiber sensor probes to binding of fluorescein molecules and gold nanoparticles. This is exploited to realize highly sensitive plasmonic fiber optic sensors.



M. Divagar, J. Saumey, Dr. J. Satija, Dr. V. V. R. Sai**