

Research Article

# Antibacterial Amphiphilic Composites of Poly(Diethylaminoethyl Methacrylate-co-Ethyl Methacrylate)/Polyindole Controlling Biofilm Adhesion for Antifouling Investigations

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**Abstract :** Amphiphilic and conductive composites are considered notable biomaterials and used as antibacterial agents because they effectively inhibit bacterial growth. In the current study; In the first stage, amphiphilic poly(DEAEMA-co-EMA) copolymers were synthesized from the hydrophilic monomer 2-diethylamino ethylmethacrylate (DEAEMA) and the hydrophobic monomer ethyl methacrylate (EMA) using free radical polymerization. In the second stage, five series composites were prepared at different concentrations using indole conductive monomer in the presence of iron(III) chloride (FeCl<sub>3</sub>) using the in situ oxidative polymerization technique in poly(DEAEMA-co-EMA) copolymer. The structures of the polymer composites (PCs) were elucidated using FTIR, TGA, SEM, AFM characterization techniques. PCs exhibited significant performance on bacterial biofilm adhesion tested using the Streptococcus mutans by the test tube method (TM). In this study, the 0.006 mg/ml concentration of PC1 reduced the biofilm formation of Streptococcus mutans by 83.199%; PC5, 89.218%; PC3 inhibited 86.078%. 0.003 mg/ml concentration of PC1 prevented S. mutans from forming biofilm by 47.055%; PC5, 71.285%; PC3 was found to inhibit +68.139%. As the concentration and amount of poly(indole) in the CPs increases, the % antibiofilm effect also increases. From these results, it can be said that PCs as biofilms may be useful materials in antifouling research.

**Keywords :** Diethylamino Ethyl Methacrylate-co-Ethyl Methacrylate, Polyindole, Antifouling, Streptococcus Mutans, Biofilm.

## 1 Introduction

Conducting polymers (CPs) constitute a class of organic compounds that are included in a large group of important commercial polymers [1]. CPs constitute a vital type of functional materials that find many comprehensive applications in science and technology [2]. Owing to their biocompatibility, the areas of use of CPs include tissue engineering [3], [4], regenerative medicine [5], [6], targeted drug delivery systems [7], [8], and cell cultivation [9]. CPs and their composites are ideal materials for the conjugation of biological equipment and electrodes in bioelectrochemical fields, because of their high conductivity [10]–[12]. CPs also serve as effective matrices in the immobilization of biological materials, realize free diffusion of substrates and substances formed in the reaction, and create a biocompatible space for microorganisms and enzymes. CPs can be prepared in different ways. One of the most suitable methods for CP production is the chemical polymerization process. The formation of polymerization is explained by three stages: The 1st stage is oxidation step, the 2nd stage is coupling and the last stage is deprotonation. With this method, very expensive experimental setups are not required for CP preparation, and at the same time, this method is scalable, so it is economical. Since there are fewer internal cross-links between polymer macromolecules, which are generally obtained by chemical synthesis, they dissolve much better in suitable solvents. Moreover, this technique is the most ideal for covalent modification of CPs. This method is the most ideal for the modification of CPs [13]. In chemical polymerization method, various oxidizing agents such as ammonium persulfate ((NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), sodium vanadate (NaVO<sub>3</sub>), cerium sulfate (Ce(SO<sub>4</sub>)<sub>2</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), potassium iodate (KIO<sub>3</sub>), potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and iron (III) chloride (FeCl<sub>3</sub>) are used [14]–[17]. Since the usage area of pure polymers is limited, their composites formed with feature conductive polymers have very widespread usage areas. Composites can be created: in situ (sequentially), ex situ (separately) and in one pot (simultaneously) [13], [18], [19]. When all techniques for the synthesis of CPs and their composites are compared, it can be seen that a wide variety of possibilities exist for the preparation of CPs and composites. Developed strategies to integrate

**Table 1: Composition of the poly(DEAEMA-co-EMA) composites with varying indole content.**

Indole content of the composite (% , m/m)	Amount of used poly(DEAEMA-co-EMA) (g)	Amount of used FeCl <sub>3</sub>		Amount of used indole		Code of Composite
		(g)	(mol × 10 <sup>2</sup> )	(g)	(mol × 10 <sup>2</sup> )	
10	3.6	1.45	0.90	0.53	0.45	PC1
30	2.8	4.37	2.70	1.58	1.35	PC2
50	2.0	7.25	4.48	2.62	2.24	PC3
70	1.20	10.20	6.30	3.68	3.15	PC4
90	0.40	13.05	8.06	4.72	4.03	PC5

CPs into a polymer matrix with effective interfacial interactions have advance developed this unique class of materials to find unlimited application. In addition, synthesis opportunities may develop towards the emergence of new composites with higher conductivity. A controlled architecture will influence the synthesis process and quality of composites in the future by tuning their structures and properties with high accuracy. It seems that polymer composite syntheses are becoming more environmentally friendly by using biological templates and using methods that place less burden on the environment. These advances in the synthesis of conducting polymers and their composites will make it possible to produce more effective and functional materials in a broad spectrum of capabilities in different fields, especially electronics, energy, and medicine.

The most used antimicrobial reagents in biomedical applications are examined in four specific groups: positive active compounds such as silver, quaternary ammonium salts and chlorhexidine, oxidants containing chlorine and peroxides, organic substances such as isothiazolones and formaldehyde, and electrophilic substances such as mercury and copper [20], [21]. In biomedical applications, the use of antimicrobial polymers with various superlatives such as chemical stability, skin impermeability and volatility is becoming more important day by day [22], [23]. Different and important antifouling functional groups such as fluoro group, quaternary ammonium group, and sulfobetaine then pave the way to make new arrangements in the molecular structure of the polymer and composite for certain end applications [24]. Amphiphilic polymers have a important superiority over cationic polymers owing to their enhanced biocidal effect opposite Gram-positive and Gram-negative bacteria [25], [26]. Microorganisms adhere to living or inanimate surfaces by creating biofilm, a polymeric substance they produce. Microbial biofilms cause serious health problems worldwide due to their ability to not affected by antibiotics, countering the host's immune response and cope with extreme environmental impact, and their relationship with established infections [27]. The development of tooth decay is relevant with biofilm formation, affecting large audiences around the world. Bacteria belonging to the *Streptococcus mutans* species are believed to be the priority etiological agent involved in this important situation. *Streptococcus mutans* plays a serious role in the formation of complex structure and multidimensional in the tooth enamel and oral mucosa [28]. It gets some cariogenic features, such as adhesion to solid surfaces, colonize the oral cavity, and they can survive in acidic conditions in the oral cavity [29]. Additionally, *S. mutans* uses acidic metabolites that produce carbohydrates, which causes acid formation and demineralization, and as a result, tooth decay occurs as a result of the removal of mineral substances [30].

In this study; for the first time, we report a facile approach to increase the antimicrobial activity against bacteria of amphiphilic and conductive composites prepared with varying amounts of hydrophilic and hydrophobic polymers. These amphiphilic composites were synthesized by in situ polymerization method. The desired antimicrobial properties were achieved thanks to the presence of the t-amine functional group in DEAEMA, which has a biofilm adhesion.

## 2 Experimental Methods

### 2.1 Materials

2-diethylamino ethylmethacrylate (DEAEMA, 99%, ethylmethacrylate (EMA, 99%), Indole (IN, 99%), Iron(III) chloride (FeCl<sub>3</sub>, 98%), ammonium persulfate (APS), Chloroform (CHCl<sub>3</sub>, 99%) (Sigma-Aldrich, St. Louis, MO, USA) was used in copolymer and polymer composite synthesis reaction.

### 2.2 Synthesis of Polymer Composites

In this study, firstly, a 50% - 50% mass ratio copolymer of DEAEMA and EMA was prepared by free radical polymerization technique in 1,4-dioxane solution with APS initiator. This prepared copolymer was used in composite preparation. Poly(DEAEMA-co-EMA)/PIN conductive composites with PIN content of 10, 30, 50, 70 and 90% by mass were prepared as stated in the method in the literature [31], and they are named with the codes PC1, PC2, PC3, PC4, and PC5, respectively. The preparation ratio of PCs are given in Table 1.

In the synthesis of PCs, firstly, FeCl<sub>3</sub> was dissolved in 25 ml of CHCl<sub>3</sub> in a three-mouthed 250 ml reaction bubble in the proportions given in Table 1. The reaction bubble was cooled to 0 °C with ice. Meanwhile, nitrogen gas was introduced into the reaction flask. Then, poly(DEAEMA-co-EMA) polymer dissolved in 25 ml of chloroform were added to this solution and mixed. In the last step, indole monomer dissolved in 25 ml of chloroform was added dropwise to the reaction mixture using a dropping funnel. The reaction was continued for 3 hours. The resulting PCs were washed with distilled water and chloroform.

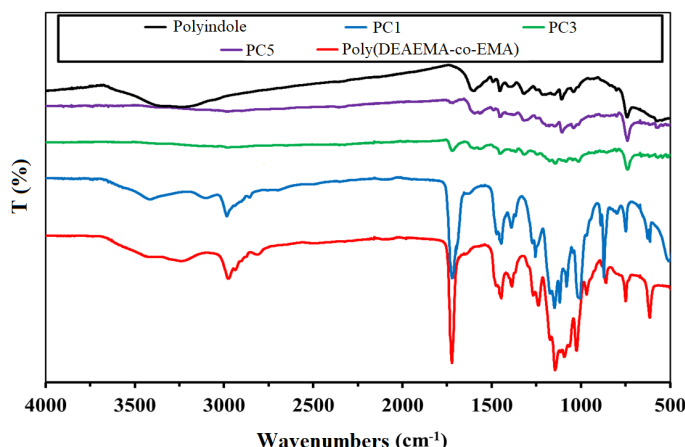


Figure 1: FTIR spectra of PIN, poly(DEAEMA-co-EMA) and PCs

Washing was terminated when the color of the washing solution became clear. The obtained PCs were filtered, dried in a vacuum atmosphere at 40 °C and stored for analysis.

### 2.3 Characterization Techniques and Measurements

Fourier transform infrared (FTIR) spectra of PCs were analyzed in the wavenumber range of 400–4000  $\text{cm}^{-1}$  with a Perkin Elmer Spectrum Two (UATR) IR Spectrometer. TGA analyzes were performed with a Hitachi 7000 TGA/DTA model thermogravimetric analyzer at room temperature – 1000°C, under a nitrogen atmosphere, at a heating rate of 10 °C/minute. Zeiss EVO LS10 model scanning electron microscope (SEM) and VEECO Multimode 8 Atomic Force Microscope (AFM) were used to examine the surface morphology of conducting polymer composites. In AFM analyses, the microscope was operated in contact mode and the force constant of the cantilever was kept at 0.15  $\text{N m}^{-1}$ . Surface scans were performed at 1 Hz.

## 3 Results and Discussion

### 3.1 FTIR Analysis

The FTIR spectra of PIN, poly(DEAEMA-co-EMA) and PCs are given in Figure 1.

In the poly(DEAEMA-co-EMA) spectrum, the C=O stretching vibration absorption peak of the ester group is seen at 1740  $\text{cm}^{-1}$ . The spectrum of poly(indole) exhibited absorption bands at 1600  $\text{cm}^{-1}$  owing to the stretching vibrations of  $-\text{C}=\text{C}-$  aromatic groups. In the spectra of PCs, absorption bands are seen at 2920  $\text{cm}^{-1}$  due to the stretching vibrations of  $-\text{CH}_2-$  and  $-\text{CH}_3$  groups [28]. PCs, the C=O stretching vibrations of the ester group of DEAEMA and EMA units exhibited bands at 1740  $\text{cm}^{-1}$  [32]. The peak at 2842  $\text{cm}^{-1}$  can be attributed to the C-H stretching vibration of the  $\text{N}(\text{CH}_3)_2$  group of DEAEMA unit [33], [34]. We can understand the stretching vibration of C-O-C of both methacrylate units from the peaks at 1150–1250  $\text{cm}^{-1}$  [35]. In each spectrum, the peak at 1020  $\text{cm}^{-1}$  indicates the C-N stretching vibration of the tertiary amine group, confirming the presence of the DEAEMA segment in the composites. The two peaks at 1380  $\text{cm}^{-1}$  and 755  $\text{cm}^{-1}$  can be attributed to the methyl group in poly(DEAEMA-co-EMA) of polymer composites. Localized signals from the C-N groups of the DEAEMA unit in the composites are seen in the other part of the spectrum at 800–730  $\text{cm}^{-1}$ . The peak at 985  $\text{cm}^{-1}$  together with the peaks at 1060  $\text{cm}^{-1}$  and 840  $\text{cm}^{-1}$ , constitutes the characteristic absorption vibration of poly(DEAEMA-co-EMA) in composites. Since there are no similar groups in the PIN unit, no intense peaks were observed in the region below 1000  $\text{cm}^{-1}$ . Based on the above discussions, it can be concluded that the prepared polymer composite is indeed macromolecular poly(DEAEMA-co-EMA)/PIN [36].

From the FTIR spectra of PCs, we can say that when the PIN ratio in the composites increases (from the PC1 to the PC5), the absorption intensity of the NH peak belonging to the PIN at 3390  $\text{cm}^{-1}$  increases. Also, In composites PC1 to PC5 concentration of poly(DEAEMA-co-EMA) decreases and accordingly the band intensity of C=O and C-N groups also decreases. DEAEMA has a hydrophilic structure and the composites retain a little moisture. This is why the broad O-H group band seen in the 3000–3500  $\text{cm}^{-1}$  region in Figure 1 is due to this. In PC5, –OH band disappeared due to very low concentration of poly(DEAEMA-co-EMA) and higher content of PIN.

### 3.2 Thermogravimetric Studies

The TGA curves of PIN, poly(DEAEMA-co-EMA) and PCs are shown in Figure 2. The results of thermogravimetric analysis (TG) is summarized in Table 2. In thermograms, the decomposition has a two-stage character; the first stage is related to the degradation of the side groups and the second is related to the degradation of the main chain. Adding different proportions

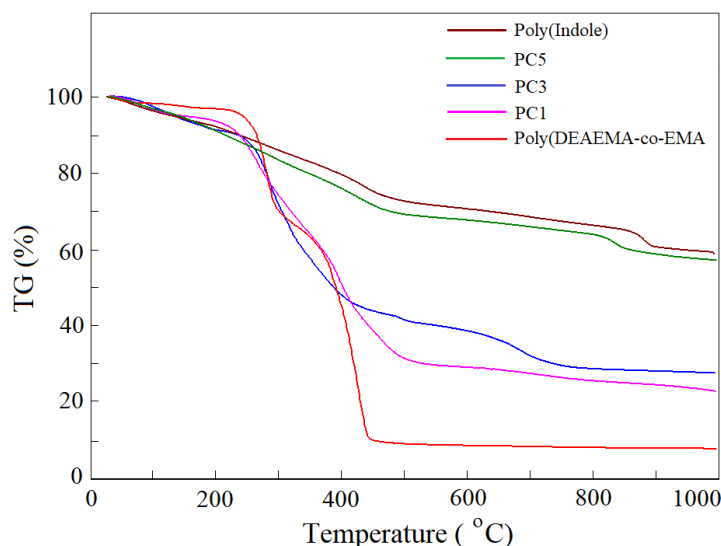


Figure 2: TGA curve of PIN, poly(DEAEMA-co-EMA) and PCs.

Table 2: Thermal analysis results of investigated compounds.

Sample name	Maximum de-composition temperature (°C)	Temperature of 40% weight loss (°C)	% Weight loss at 400 °C	% Weight loss at 700 °C	% Residue at 1000 °C
Poly(DEAEMA-co-EMA)	430	416	51	90	9
PC1	468	420	44	71	24
PC3	642	394	48	64	32
PC5	805	872	23	30	65
Poly(Indole)	830	908	16	27	67

of DEAEMA, EMA and Indole to composites can significantly reduce or improve thermal resistance [37]. In order to obtain complete information about the course of thermal degradation, polymer composite samples were heated at various temperature ranges. The most in-depth studies of the thermal properties of PDEAEMA were published in [38]. This decomposition occurred in two steps. It was confirmed that in the first phase of the first stage of decomposition, the amino groups undergo thermal destruction, and in the second phase, the disappearance of side groups can be observed. The first stage occurred in the 290–400°C range; and the second is located between 390–550°C.

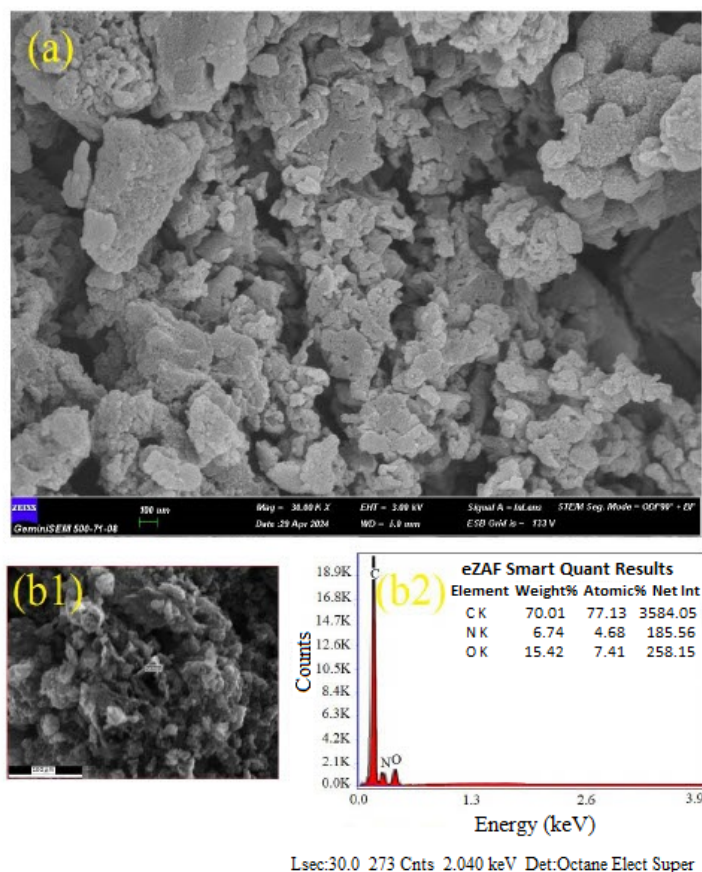
Different proportions of DEAEMA-EMA copolymer and Indole were used in the composites we prepared. Since the order of DEAEMA and EMA units and the order of Indole units in the composites may be different, the thermogram curves of the composites may differ even though they are similar to the thermograms of their homopolymers. For this reason, the 40% weight loss temperature and the % mass loss values at 400°C of the PC3 sample did not show a similar degradation curve with the other composites. It is likely that the order and amount of DEAEMA-co-EMA units in PC3 in the composite composition differed slightly from other composites.

It can be seen from the TG curves that when the PIN ratio in PCs increases, the residue release rate of the composites also increases. It is observed that PCs with high poly(DEAEMA-co-EMA) ratios have 40% mass loss around 400°C and show more stable behavior. It is seen that PCs are stable at higher temperatures (700-1000°C) as the PIN ratio increases and leave residue at rates of 40-47%. It can be said that this is caused by the 6-ring and 5-ring groups in PIN. It is reported in the literature that the decomposition temperature of PIN is higher than the values reported for conductive polymers such as polyaniline, polythiophene, and polypyrrole [39].

### 3.3 Morphological Analysis

It is an emphasized fact that the features of materials depend largely on their morphological structures. In terms of physicochemical and biological properties, the distribution of components in polymer composites is extremely important. FESEM images of PC4 has been seen in Figure 3(a). As can be seen FESEM image, we can said that, PC4 has a ununiform, unhomogenous and sponge-like structure. The surfaces on which FESEM EDX field spectra and EDX analysis was performed in the PC4 is seen in Figure 3(b). The peaks of C, N and O elements were viewed 0.275; 0,410 and 0,530 keV, respectively.

At the same time, a microscopic examination of PC4 was also examined using AFM. Examination was performed with the surface using contact mode. In the study, the typical force constant of the console was kept constant at 0.15 Nm<sup>-1</sup>. The surface



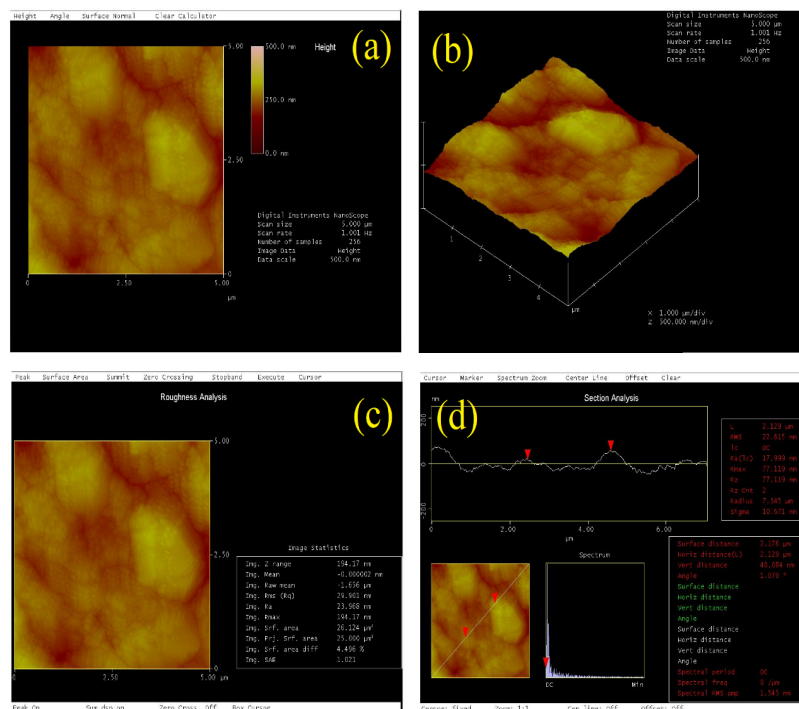
**Figure 3: (a) FESEM and (b1-b2) EDX field analysis of prepared PC4.**

morphology examination of PC4 was carried out at a scan rate of 1.00 Hz [Figure 4(a)]. A typical three-dimensional (3D) image and light colors are seen at elevations in Figure 4(a)'s AFM images, dark colors are seen at depths of PC4 in AFM are shown in Figure 4(b). It can be seen that PC4 has a cloudy form in the AFM image. By selecting a horizontal section as the profile, a 2D AFM image ( $5\ \mu\text{m} \times 5\ \mu\text{m}$ ) cross-section of PC4 were obtained. In Figure 4(d) indicates its RMS values are (22.615 nm), (40.084 nm) distance from this data; we say that distance: (2.119  $\mu\text{m}$ ) vertical distance: (4048 nm) out-of-plane Distance : (276 nm), horizontal distance: (5  $\mu\text{m}$ ), and patchy and unhomogeneous structure on the bare glass surface.

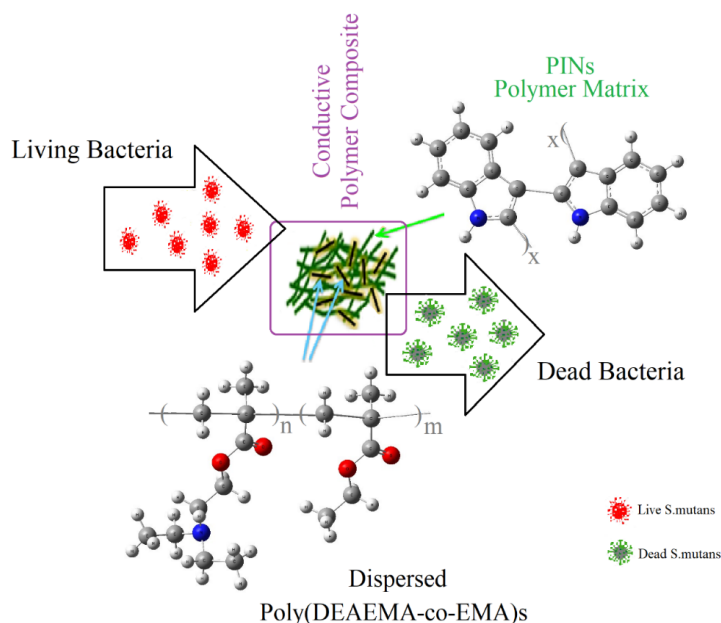
### 3.4 Detection of Antibiofilm Effect

Polymer/polyindole conductive polymer composites are gaining attention owing to their potential applications in different fields, including biomedical and antibacterial coatings. The surface properties of the composite play a crucial role in preventing bacterial adhesion and subsequent biofilm formation. Biofilms are colonization of living or non-living surfaces by adhering to them through the exopolysaccharides they produce. Properties such as surface roughness or smoothness, hydrophobicity, chemical composition and surface charge determine the extent of adhesion. Positively charged and hydrophilic surfaces were more selective towards electroactive microbes and more conducive to electroactive biofilm formation [40]. Amphiphilic copolymer of poly(DEAEMA-co-EMA) are partially soluble in water, poly(DEAEMA) is water soluble and poly(EMA) is water insoluble due to hydrophilic and hydrophobic nature. These amphiphilic copolymers played profound effect on the antibacterial activity. Amphiphilic composites of poly(DEAEMA-co-EMA)/PIN have shown different zones of inhibitions along side Gram-positive bacteria *Streptococcus mutans*. PC1 has more concentration of poly(DEAEMA-co-EMA); hence it is proved higher activity against bacteria owing to it had higher charge density owing to amino groups [41]. Because, the Gram-positive bacteria have cell wall that made up of only peptidoglycan the diffusion for the cationic polyelectrolytes with hydrophobic group are easier [42]. The presence of polyindole in the composite can change the surface energy and topography, making it suitable for bacterial attachment. Polyindole, a conducting polymer, can exhibit electrochemical activity. The composite can be designed to incorporate antimicrobial agents or drugs into its matrix. Poly(diethylaminoethylmethacrylate-co-ethylmethacrylate), a polymer with diethylamino groups, can serve as a matrix for the controlled release of these substances. This sustained release inhibits





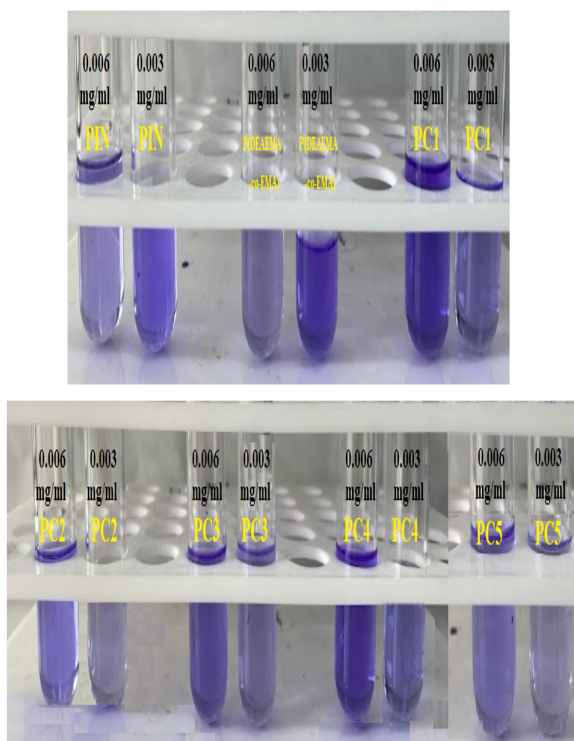
**Figure 4: (a) 2D AFM image (b) 3D AFM image (c) Roughness analysis (d) Cross section analysis of the PC4 coated glass. (The scan size is  $5\ \mu\text{m} \times 5\ \mu\text{m}$ ).**



**Figure 5: Schematic illustration of composites that control bacterial adhesion.**

bacterial growth and biofilm formation. Due to the antifouling property of DEAEMA, it has the ability to repel and destroy microorganisms, and EMA has the antibacterial property [43]. Polyindole can exhibit pH-sensitive behavior such as swelling or shrinkage in response to changes in pH. This property can be exploited to create surfaces that undergo structural changes in response to bacterial colonization and inhibit biofilm formation. Schematic illustration of composites that control bacterial adhesion is given in Figure 5.

Pathogens were planted in Nutrient Broth medium and incubated at  $37\ ^\circ\text{C}$  for 24 hours. After incubation, cultures were adjusted to 0.5 McFarland turbidity ( $10^6\ \text{CFU mL}^{-1}$ ).  $200\ \mu\text{g}$  of *Streptococcus mutans* was inoculated into the previously



**Figure 6: Crystal Violet color developed by some Streptococcus mutans isolates showing different dyeing process of their formed biofilms as determined by test tube method.**

**Table 3: Inhibition of biofilm formation (%).**

Tested polymers and composites	30 mg/ml concentration	60 mg/ml concentration
Poly(Indole)	73,695	90,495
Poly(DEAEMA-co-EMA)	42,838	81,058
PC1	47,055	83,199
PC2	63,253	85,743
PC3	68,139	86,078
PC4	68,495	86,546
PC5	71,285	89,218

prepared Nutrient Broth medium containing test material at 30 and 60  $\mu\text{g}$  concentrations. 0.5 McFarland was added to these tubes and then were incubated at 37  $^{\circ}\text{C}$  for 24 hours. After incubation, the liquid medium was poured out and the tubes were washed 3 times with distilled water. First, ethanol: acetic acid (95:5) was added to the dried tubes and fixed for 10 minutes, and at the end of the period, the tubes were emptied and left to dry. Then, 0.1% crystal violet was added to the dried tubes and the dyeing process was performed. The results are seen in Figure 6. After the dyeing process, the crystal violet in the tubes was emptied, washed 2-3 times with distilled water, and then dried. To remove the stained and attached bacteria in the drying tubes, ethanol: acetic acid (95:5) was added to the wells and incubated for 10 minutes. The dye was allowed to dissolve by waiting for a while. At the end of the period, the optical density value of the microplate was measured at 570 nm in a spectrophotometer and the biofilm percentages were calculated compared to the positive control [44]. Inhibition of biofilm formation (%) was calculated from the equation below, and the results are given in Table 3.

$$\text{Inhibition \%} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

where:

- A control: Absorbance value containing only the bacterial strain
- A sample: Absorbance value with added test material

Antibacterial activities of all composites with different concentrations of poly(DEAEMA-co-EMA) were assessed against Gram-positive bacteria which commonly cause biofilm on materials [21]. Biofouling resistance of synthesized composite materials against *Streptococcus mutans* biofilm formation was studied. Antibacterial activity also influenced by the spacer length owing to conformation charge density on the composites [24]. As well as DEAEMA monomer had positive charge because ammonia group copolymerized and EMA monomer had no charge with hydrophobic in nature. The increase in antibacterial activity increase with charge density by polymerization and assumed more adhesion owing to negative charged bacterial cell surface, increase dispersal through the cell wall. These cationic polymers fix to the cytoplasmic membrane, interruption the cytoplasmic membrane, discharge of intracellular elements and bacterial cells mortality [45]. Composites antibacterial action mechanism occurred by (a) bacterial cell surface adsorption, (b) cell Wall diffusion, (c) cytoplasmic membrane adsorption, (d) cytoplasmic disruption, (e) leakage of cellular components, and (f) cell death [46]. Here more positive charge present on composites PC1 and PC2 that caused interactions between composites and bacteria. So it is a critical factor, and further action disrupted the cell wall, fluid leaked, cell ruptured and death occurred [47].

#### 4 Conclusion

Biofilms are considered microbial cells in an unusual structure. Proliferation of microbial cells occurs within the polymeric matrix, which acts as a protective coating. Biofilms contribute to the character of antimicrobial resistance. The fact that the behavioral mechanism of biofilms under different environmental conditions has not been sufficiently elucidated in research shows that there are inconsistencies between in vitro analysis results and in vivo studies. In this study, we aimed to prepare a polymer composite with biofilm-forming properties that can be used to remove harmful dyes. For this purpose, we obtained a series composite by polymerizing the indole conductive polymer with the in situ polymerization method in the poly(DEAEMA-co-EMA) polymer we prepared. We elucidated the chemical structure of this molecule using spectroscopic methods and characterized its surface morphology. *Streptococcus mutans* is a gram-positive, facultative anaerobic bacterium mostly found in the human oral cavity and is an important factor in the formation of dental caries. Here, we observed for the first time that the polymer composite we prepared created a protective biofilm effect against this bacteria.

#### Authors' Contributions

CS: Sources, Research, Formal analysis, Validation, Methodology, Visualization, Writing – Original Draft. BT: Sources, Research, Formal analysis, Validation, Methodology, Visualization, Writing – Original Draft.

#### Competing Interests

The authors have no conflicts of interest to declare regarding the content of this article.

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