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Poly(hexamethylene biguanide) immobilized non-absorbable and antimicrobial PET fiber for surgical suture applications: synthesis, characterization and *in vitro* cytocompatibility assessment

Cerrahi sütür uygulamaları için poli(heksametilen biguanid) immobilize edilmiş emilmeyen ve antimikrobiyal PET fiber: sentez, karakterizasyon ve *in vitro* sitouyumluluk değerlendirmesi

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Abstract

Surgical sutures are medical devices commonly used to close wounds. However, surgical site infections (SSI) are highly likely to occur around the suture after surgery. In this study, we aimed to synthesize a multi-filament polyethylene terephthalate (PET) based non-absorbable antimicrobial suture to prevent possible CAI. PET fiber was grafted with methacrylic acid (MAA-g-PET) to give it a functional group. MAA-g-PET fibers were immobilized with poly(hexamethylene) biguanide (PHMB), a cationic antiseptic with broad spectrum antibacterial and antifungal activity (PHMB/MAA-g-PET). The synthesis of PHMB/MAA-g-PET fibers was confirmed by SEM, FTIR, DSC and TGA characterizations. The antimicrobial activity of PHMB/MAA-g-PET fiber was investigated against *S. aureus* and *E. coli* species and proved to be >99% effective against both species. MTT cytotoxicity assay revealed that PHMB/MAA-g-PET fiber was cytocompatible for L929 fibroblast cells. In addition, *in vitro* degradation studies showed that the degradation profile of PHMB/MAA-g-PET was not much different from the original PET and retained its non-absorbable property.

Key Words

"Antimicrobial suture, non-absorbable suture, polyethylene terephthalate, polyhexanide, polyhexamethylene biguanide, surgical suture"

Öz

Cerrahi sütürler yaraları kapatmak için yaygın olarak kullanılan tıbbi cihazlardır. Ancak cerrahi sonrasında sütür çevresinde cerrahi alan enfeksiyonlarının (CAE) ortaya çıkma olasılığı yüksektir. Bu çalışmada olası CAE'nın önüne geçmek için multi-filament polietilen teraftalat (PET) tabanlı emilmeyen antimikrobiyal iplik sentezlenmesi amaçlanmıştır. PET life metakrilik asit (MAA-g-PET) aşılanarak fonksiyonel grup kazandırılmıştır. MAA-g-PET lifler geniş spektrumlu antibakteriyel ve antifungal aktiviteye sahip katyonik bir antiseptik olan poli(heksametilen) biguanid (PHMB) immobilize edilmiştir (PHMB/MAA-g-PET). PHMB/MAA-g-PET liflerin sentezlendiği SEM, FTIR, DSC ve TGA karakterizasyonları ile doğrulanmıştır. PHMB/MAA-g-PET ipliğin antimikrobiyal aktivitesi *S. aureus* ve *E. coli* türlerine karşı araştırılmış ve her iki türe karşı >%99 etkili olduğu kanıtlanmıştır. MTT sitotoksisite deneyi PHMB/MAA-g-PET ipliğin L929 fibroblast hücreleri için hücreuyumlu olduğunu ortaya koymuştur. Ayrıca *in vitro* degradasyon çalışmaları PHMB/MAA-g-PET'in degradasyon profilinin orijinal PET'ten çok farklı olmadığını ve emilemez özelliğini koruduğunu göstermiştir.

Anahtar Kelimeler

"Antimikrobiyal sütür, emilmeyen sütür, polietilen tereftalat, poliheksanid, poliheksametilen biguanid, cerrahi sütür"

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1. Introduction

Surgical site infections (SSIs) are infections that develop within 30 days of surgery or within one year of a medical device being inserted, despite the progress made in modern healthcare. Surgical site infections (SSIs) have a substantial influence on the occurrence of illness, extended hospital stays, and death. An infection at the surgical wound site not only poses a risk to the intended healing of the wound, but can also progress into life-threatening situations, particularly in patients who are critically unwell (Ercan et al., 2018). Medical devices, such as catheters, stents, sutures, and bone scaffolds, are responsible for almost 60% of infections that occur in hospitals (Anjum et al., 2017). These medical devices, which are foreign to the body, have the potential to create bacterial bioadherence and accumulation of microbial colonization in wound incisions (Vinh & Embil, 2005).

Even with the exponential growth of medical technology, surgery remains an essential component of contemporary medicine. Sutures have been used to close surgical incisions since 3500 BC, and they remain the most widely used approach even with the advent of new techniques like surgical staples. Sutures are an essential component of surgical treatments. A surgical suture is a filamentous medical device used to keep open ends of wounds together. In order to offer sufficient mechanical support for wound closure, a surgical suture must be able to sustain typical physiological levels of mechanical stress (Kjaergard, 2001; Marsidi et al., 2020). Based on the thread type and biodegradability, sutures can be categorized as either non-absorbable or absorbable. They can also be categorized as monofilament or multifilament (or braided) (Grigoras et al., 2016).

Closing surgical wounds requires the use of sutures. But like other medical implants, they are foreign objects at the site of the wound and serve as a nidus for the adhesion of bacteria. Thus, the presence of sutures dramatically (about 10,000-fold) raises the risk of infection at the surgical site as well as the sensitivity of a wound to infections. Furthermore, when bacteria colonize the suture surface, their sensitivity to antibiotics is significantly decreased (Storch et al., 2004). The properties sought in an ideal surgical suture material can be listed as follows (Coşkun et al., 2016): Its smooth surface and easy-to-tie structure are essential. It should also be elastic, pass through tissue without causing damage, exhibit sufficient tensile strength until healing is completed, have knot reliability and low memory ability, not be absorbed before the wound heals, be absorbed as soon as healing is complete, be easy to sterilize, cause minimal reaction in the tissue, be free of allergies and carcinogens, and not allow bacterial growth and infection. The complicated process of biofilm formation on surgical sutures starts with pathogen adherence to the suture material. For biofilm formation and the subsequent development of suture-associated SAI, bacterial adherence into the surgical wound during the first four to six hours after suture implantation is essential. Furthermore, the most significant virulence component of *S. aureus* and *E. coli* is said to be the initial bacterial attachment to a foreign substance during the biofilm development process. Consequently, it is thought that a crucial intervention for the prevention of suture-related SAI is the prevention and/or inactivation of initial bacterial adhesion as well as the inactivation of easily adhering bacteria soon after surgical suture placement. In addition to virulence factors of pathogens, the physical properties and chemical composition of suture materials have an interrelated effect on bacterial adhesion (Masini et al., 2011).

Chu et al. discovered that the absorbability of various suture materials varies with the type of bacteria they encounter. Additionally, they demonstrated that bacteria can adhere to the surface of Dexon® (polyglycolic acid) sutures (Chu & Williams, 1984). Imaging methods have also demonstrated that polyglycolic acid sutures exhibit superior bacterial adherence compared to monofilament polypropylene sutures. Furthermore, compared to monofilament sutures, braided sutures are easier to conceal bacteria from view, making them more favorable for bacterial colonization. Because sterile silk sutures are made of protein, which can act as a nutrient for bacterial growth, some research has indicated that they can greatly increase bacterial colonization and retention. In order to reduce suture-induced SAIs, researchers have created a variety of functional medical sutures by combining medications, which may include various antimicrobial agents or drug delivery systems (Li et al., 2021; Raut, 2019).

The primary strategy for preventing wound infection is to modify surgical sutures by coating or impregnating them with antibacterial chemicals. According to research by Alexender et al., antibacterial surgical sutures can lower the risk of infection in the wound area after surgery by preventing bacterial colonization (Alexander et al., 2011). Moreover, surgical sutures can be used in surgical applications such as wound closure, reconstructive surgery, bone surgery, strabismus surgery, tendon repair, minimally invasive surgery to internal organs, cosmetic and cervical correction with physical and chemical modifications (Phan et al., 2021).

Many researchers have developed sutures containing various antimicrobial agents such as triclosan, chlorhexidine, sulfamethoxazole trimethoprim, tetracycline hydrochloride and levofloxacin. In addition, chitosan, silver nanoparticles, antimicrobial peptides and grapefruit seed extracts have also been used to produce antimicrobial surgical sutures (Li et al., 2021). However, some antimicrobial agents lose their effectiveness with the development of bacterial resistance. Triclosan-coated antimicrobial sutures have been developed and are commonly employed to prevent bacterial adhesion to the suture surface and reduce the risk of surgical site infections. Triclosan is an antibacterial chemical that has demonstrated strong activity against both gram-positive and gram-negative pathogens. Nevertheless, the improper utilization of triclosan in soap and its widespread application in antimicrobial sutures have led to the activation of efflux pumps and the deterioration of enzymes, among other processes. These factors have played a role in the development of bacterial resistance. Triclosan bioaccumulation has been reported in human milk, adipose tissue, urine, and umbilical cord blood. Furthermore, there is evidence suggesting that triclosan bioaccumulation in the human body may have detrimental impacts on the immunological, endocrine, and reproductive systems (Rees Clayton et al., 2011).

Antimicrobial agents, such silver nanoparticles (AgNPs), have been utilized in the creation of antimicrobial sutures to combat SAI by effectively targeting a wide range of germs. Nevertheless, the mechanisms by which topography affects bacterial adherence and the extent to which silver promotes tissue compatibility are still not well comprehended and substantiated (Yang et al., 2017). Chlorhexidine-coated sutures have been used as an alternate method to prevent surgical site infections (SAI) because they have a wide range of effectiveness against various types of microorganisms. Nevertheless, the effectiveness of chlorohexidine-coated sutures in reducing surgical site infections (SAI) may be hindered by their short-term antibacterial action and low biocompatibility (Obermeier et al., 2015). Thus, there is a need for a novel and efficient suture that can hinder the first attachment of pathogens to the surface of the suture in order to reduce suture-associated surgical site infections (SAI).

Poly (hexamethylene biguanide) hydrochloride (PHMB, polyhexanide) (Figure 1) is a positively charged substance used as a biocide. It is widely sold globally because of its capacity to effectively kill bacteria, viruses, and fungi. Additionally, it is chemically stable, has minimal toxicity, and is reasonably priced (De Paula et al., 2011; Mashat, 2016; Yılmaz & Akın, 2022). PHMB interacts with the negatively charged phosphate head groups of phospholipids in the bacterial cell wall, leading to enhanced stiffness and the insertion of non-polar sections into hydrophobic regions. This disrupts the membrane and causes deterioration of the cytoplasm, ultimately ending in the death of the cell (Mashat, 2016). Put simply, the antibacterial effect of a positively charged PHMB is due to the interaction between the cationic amino groups in the PHMB molecule and the anionic phospholipids in the bacterial cell wall (Zhao & Chen, 2016).

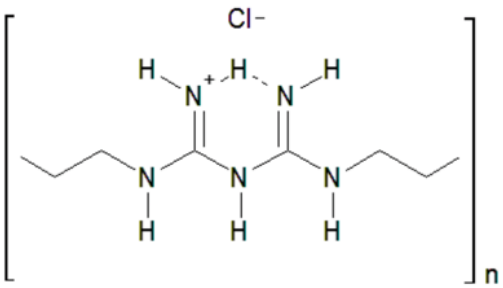


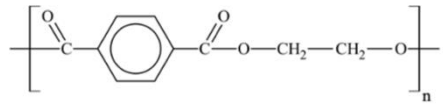
Figure 1. Chemical structure of the PHMB repeating unit.

There have been no known instances of microorganisms developing resistance to PHMB. Topical application of PHMB is well tolerated on the skin, eyes, ciliated epithelium of the nose, and wounds. The market for goods containing PHMB, including liquids, gels, disinfectants, and antimicrobial dressings, has shown significant growth in recent years. Furthermore, PHMB finds use in various industries such as swimming pool disinfectants, cosmetics, skin protectants, contact lens disinfectants, agricultural and food processing (cleaners), fibers, textiles, treatment of hatching eggs, and technical fluids like cutting oils and adhesives (Kaehn, 2010). In their study, Koburger et al. conducted a comparison of the antiseptic effectiveness of triclosan, octenidine, PHMB, PVP-iodine, and chlorhexidine digluconate. They found that PHMB is the most appropriate antiseptic for situations where prolonged use or prolonged contact is necessary, as well as for treating chronic wounds, due to its superior tolerability (Koburger et al., 2010). According to the present research, it has been found that PHMB can be safely added to surgical sutures at an optimal amount. Recent research has explored different methods, such as coating, grafting, electrospinning and blending, to incorporate natural or synthetic antiseptic chemicals into suture devices. The goal is to create surgical sutures with antimicrobial properties (Li et al., 2021).

Surgical sutures are categorized as absorbable and non-absorbable based on their intended purpose (Avgoustakis, 2005; Chu, 2013). Silk, ultra-high density polyethylene (UHDPE), polypropylene (PP), polyvinylidene difluoride (PVDF), polyamide (PA), poly(tetrafluoro ethylene) (PTFE) and PET are commonly used non-absorbable suture materials. Poly caprolactone (PCL), poly(glycolic acid) (PGA), polylactide (PLA), poly(glycolide-co-caprolactone) (PGCL), poly(lactic-co-glycolide acid) (PLGA), poly(glycolide-co-lactide) (PGLA) and polydioxanone (PDO) are some of the absorbable sutures.

PET sutures are non-absorbable medical devices made of polyethylene terephthalate polymerized from ethylene glycol and terephthalic acid (or dimethyl terephthalate (Figure 2) (Chu, 2013). PET is a highly utilized engineering polymer because of its exceptional mechanical qualities, great resistance to corrosive substances such as acids and oxidizing agents, ability to withstand sunlight and microbes, and its cost-effective manufacture (Bozkaya et al., 2012; Coşkun et al., 2017). The application areas of this product include cosmetics, apparel, biomedical materials, food packaging materials, and other fields. Furthermore, PET, which is manufactured as a non-absorbable polyester suture and marketed as TR0-POLYFIL by Troge Medikal in Germany, finds application in various surgical fields such as orthopedics, neurology, cardiovascular surgery, ophthalmology, gastrointestinal surgery, and skin closure procedures.

(a)



(b)

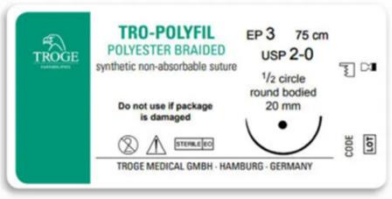


Figure 2. (a) Chemical structure of poly (ethylene terephthalate) polymer, (b) Commercial monofilament PET surgical suture.

PET is hydrophobic and does not contain chemically reactive groups. Therefore, many functional groups such as carboxylic acid, amine, hydroxyl, and epoxy groups can be introduced to the PET surface by using different monomers with various methods. PET fibers with functional groups have been used for many purposes such as removal of metal ions and acidic-basic dyes from aqueous solutions, enzyme immobilization, antimicrobial activity (Temoçin & Yiğitoğlu, 2009; Ünlü et al., 2020). Since PET alone has no antimicrobial effect, many studies have been conducted to incorporate antibacterial and antifungal agents into the structure of PET, especially for use in healthcare. Arslan and Gunay grafted 4-VP, MMA and GMA to PET fibers by graft copolymerization method. By introducing various groups such as amine, chlorine, hydrogen peroxide and triclosan into the grafted PET fibers, they proved the antibacterial activity of modified PET fibers against *E. coli* and *S. aureus* species (Arslan & Günay, 2019). Gün Gök et al. prepared PET fiber with amine functional group by chemically reacting hexamethylenediamine with MAA grafted PET fibers. They then investigated the adsorption of silk sericin-coated silver nanoparticles onto the modified PET material and evaluated its antibacterial activity against *E. coli* and *S. aureus* species (Gün Gök et al., 2020). Anjum et al. conducted a study where they immobilized bioactive nanogels and chlorhexidine on the surface of poly (ethylene terephthalate) filaments with carboxyl functional groups using plasma and chemical reaction methods. The researchers found that the suture showed a specific level of antibacterial activity (Anjum et al., 2020).

2. Materials and Methods

2.1. Material

The PET material, which has a linear density of 122 decitex and is made up of multiple filaments sourced from SASA in Turkey, underwent a reflux process with acetone for a duration of 12 hours to eliminate any potential impurities. Subsequently, it was dried in a vacuum oven at a temperature of 50 °C until a constant weight was achieved. 20% PHMB solution was purchased from Ataman Chemical (Turkey).All additional chemicals were utilized in a state of high purity, sourced from Sigma-Aldrich.

2.2. Preparation of PET Fibers for Grafting

A sample of PET fiber, weighing 0.3±0.01 g, was subjected to a 6-hour washing process using acetone in a sokslet apparatus. Afterward, it was dried until a constant weight was achieved and then transferred into 100 mL Pyrex tubes. The fibers underwent swelling in a solvent called 1,2-dichloroethane (DCE) at a temperature of 90 °C for a duration of 2 hours. After the swelling procedure concluded, the surplus solvent on the yarn was eliminated using filter paper and promptly immersed in the solution medium for graft copolymerization.

2.3. Immobilization of PHMB on PET fibers functionalized with MAA

Initially, PET fibers were introduced into a polymerization tube with a capacity of 100 mL. Subsequently, methacrylic acid (MAA) was used as a monomer to impart a functional group to the PET fibers. Next, 2 mL of benzoyl peroxide dissolved in acetone was introduced into the solution. The resulting combination was then brought up to a total volume of 20 mL by adding water. Immediately after, the mixture was placed in a water bath set to a temperature of 80 °C. Following a duration of 2 hours, fiber samples extracted from the polymerization mixture were rinsed with distilled water. Following a 12-hour Soxhlet extraction using water to eliminate ungrafted monomers/polymers, the samples were subsequently subjected to drying in an oven at a temperature of 37 °C until a constant weight was achieved. The grafting efficiency (% GE) was then calculated gravimetrically from the original and grafted fiber masses using the following equation (1):

$$\% \, \boldsymbol{GE} = \frac{\boldsymbol{w_g - w_0}}{\boldsymbol{w_0}} \boldsymbol{x100} \quad \textbf{(1)}$$

where w_o : mass of the original fiber (g), w_g : mass of the grafted fiber.

For PHMB immobilization, MAA grafted PET fibers (MAA-g-PET) were placed in 20% (w/w) PHMB solution and stirred at 85 ºC for 12 hours. At the end of the experimental period, the solution was washed with distilled water to remove excess PHMB. It was then dried in an oven at 37 ºC until constant weight and then characterized. A schematic illustration of the experimental stages is given in Figure 3.

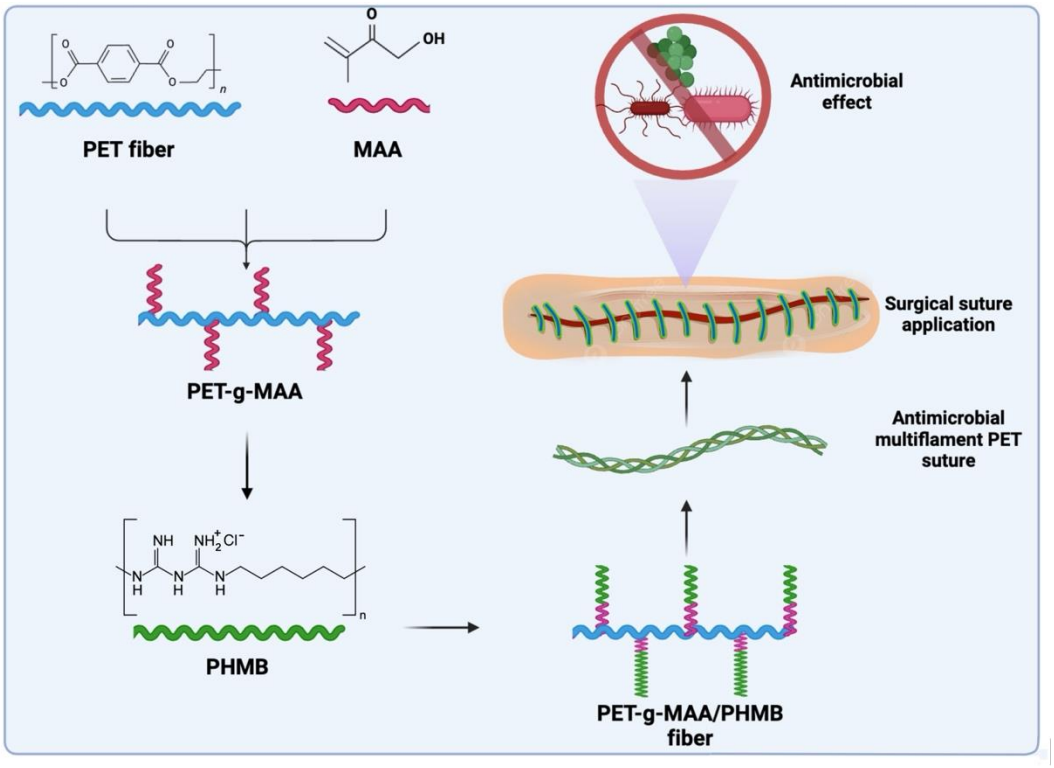


Figure 3. Schematic illustration of the synthesis steps and application of PET-g-MAA/PHMB material.

2.4. Characterization

2.4.1. SEM Analysis
Original PET and modified PET fibers were analyzed by Scanning Electron Microscope (SEM, Jeol JSM 5600, Tokyo).

2.4.2. FTIR Analysis
Functional group analysis of the materials was performed by Fourier transform infrared (FTIR) spectroscopy (Bruker Vertex 70 V, USA) using attenuated total reflectance (ATR) assignment according to ASTM E1252 (scan rate: 32 cm-1, resolution: 4 cm-1).

2.4.3. DSC Analysis
The thermophysical properties of PET, MAA-g-PET, and PHMB/MAA-g-PET materials were investigated using a differential scanning calorimeter (DSC, Q 2000, TA Instruments, USA). The analysis was conducted at a heating rate of 20°C/min in a nitrogen gas (N2) atmosphere, using an aluminum T-zero hermetic pan.

2.4.4. TGA Analysis
The study utilized a thermogravimetric analyzer (TGA) (TA, Q 500) to ascertain the sequential mass reduction of the materials in relation to temperature. The materials underwent analysis using a ceramic pan in an environment of nitrogen gas (N_2) with a heating rate of 20°C per minute till reaching a temperature of 600°C.

2.4.5. Contact Angle Analysis
A video camera-based CAM 200 Contact Angle Measurement Device (KSV Instruments Ltd., Finland) was used to determine the water contact angles of PHMB modified MAA-g-PET fibers. The contact angle of the material prepared as a disk with a diameter of 10 mm was measured by sessile drop method at room temperature by dropping ultrapure water (0.8 µL) for 10 seconds.

2.4.6. In vitro Degradation of PHMB/MAA-g-PET Material

The sutures that were manufactured underwent an in vitro degradation test, which followed the guidelines outlined in ASTM F1635-16. Prior to the experiment, the weights of the fibers, which were cut to a length of 20 mm, and the weights of the falcon tubes in which they were placed were measured and recorded. The specimens were thereafter placed in containers with 50 mL of PBS (pH 7.4) solution and subjected to incubation at 37 °C in an oven. At specific time intervals, the fluids in the flasks were moved to other tubes. The experimental phalcons and nanofibers within were then dried together under vacuum and measured in terms of weight. The sutures' mass loss (ML) at specific time intervals was determined by applying the following equation (2):

$$ML(\%) = \frac{W_0 - W_t}{W_0} \quad (2)$$

where w_0 is the initial sample weight and w_t is the weight of the sample taken at a given time.

2.4.7. Antimicrobial Activity Test

A minimal inhibition concentration (MIC) method was used to determine the antimicrobial activity of PHMB/MAA-g-PET material on *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacterial strains and the experimental protocol was reported in our previous study.*(O. Bozkaya et al., 2023).*

2.4.8. In vitro Cytocompatibility Test

The cytotoxic level of PET-g-MAA/PHMB material designed as a surgical suture was investigated in an accredited in vitro biocompatibility laboratory according to the MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) test protocol specified in TS EN ISO 10993-5 Standard (E. Bozkaya et al., 2023). L929 fibroblast cell line was used for MTT assay.

3. Results and Discussion

3.1. Morphological Analysis

SEM images of the original and modified PET fibers are shown in Figure 4. When the photomicrographs of the original PET fiber (Figure 4a) and MAA-g-PET fiber (Figure 4b) are examined, the smooth surface of the original PET fibers became relatively rough after MAA grafting and the fiber diameter changed. On the surface of MAA-g-PET fibers treated with PHMB, it is seen that the heterogeneity increased slightly more than MAA-g-PET fiber (Figure 3c). Therefore, these changes on the surface of the fibers increase the possibility of morphological modification. In addition, it is clearly seen in Figure 4d that there is no damage such as breakage, rupture etc. in the fibers of PET yarn with multifilament structure after modifications.

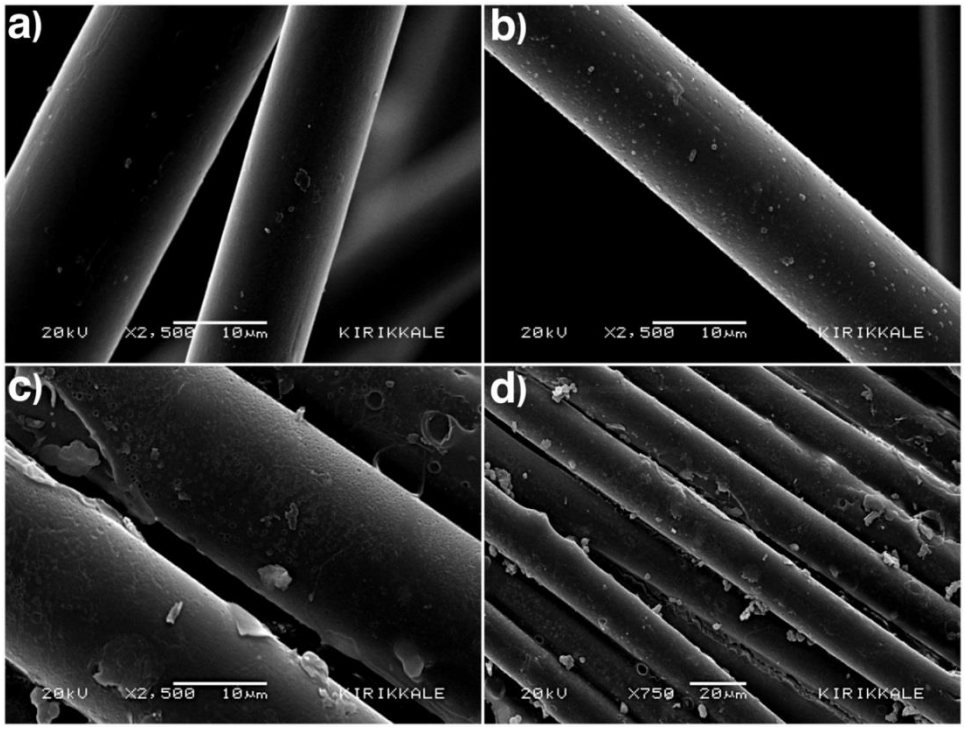


Figure 4. SEM micrographs of **(a)** original PET, **(b)** PET-g-MAA (graft yield: %56), **(c)** PHMB/PET-g-MAA and **(d)** multifilament PHMB/PET-g-MAA materials.

3.2. Chemical Bond and Functional Group Analysis of Original and Modified PET Materials

Figure 5 displays the FTIR spectra of the original PET, PHMB, MAA-g-PET, and PHMB/MAA-g-PET fibers. The peaks observed at 1410 cm^{-1}, 1711 cm^{-1}, and 2963 cm^{-1} in Figure 5a represent the distinctive absorbance bands of PET fiber. These peaks correspond to the stretching vibrations of aromatic C=C, C=O, and C-H bonds, respectively. The introduction of MAA into PET fibers through grafting results in broadening of the band at 1711 cm^{-1} in PET-g-MAA fibers. This broadening is caused by the presence of C=O groups in the structure of the grafted MAA, as seen in Figure 5b. The observed alteration in the spectrum provides evidence of the grafting of MAA groups onto PET fibers (Gün Gök et al., 2021). In addition, a wide range of frequencies between 3000 and 3500 cm^{-1} exhibited OH stretching peaks, which were caused by the presence of carboxyl acids from MAA monomers that were grafted onto the PET structure. The absorption peak at 2989 cm^{-1} is caused by the stretching of CH bonds in the C-CH_3 compounds of grafted MAA. The observed alterations in the FTIR spectra of PET-g-MAA fibers provide evidence of the successful grafting of MAA monomers onto the PET surface through a radical polymerization process. The absorption peaks observed at 3300 cm^{-1}, 1634 cm^{-1}, and about 1552 cm^{-1} in the PHMB spectra (Figure 5c) can be ascribed to the stretching of N-H bonds, stretching of C=N bonds, and bending vibrations of NH_2^+ groups, respectively (Dilamian et al., 2013). The spectrum of MAA-g-PET fibers treated with PHMB (Figure 5d) clearly shows the presence of distinctive bands of PHMB in the ranges between 3000-3500 cm^{-1} and 1500-1700 cm^{-1} (Liang et al., 2020). FTIR analysis indicates that PHMB/MAA-g-PET fibers were successfully produced.

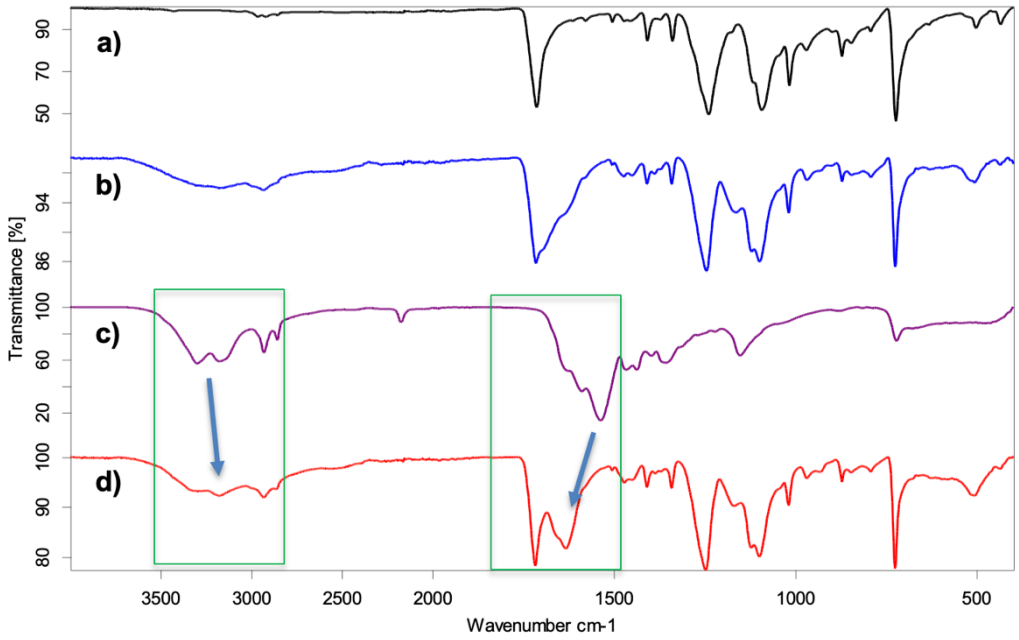


Figure 5. FTIR spectra of (**a)** original PET fiber, (**b)** MAA-g-PET (graft yield: %56), (**c)** pure PHMB, (**d)** PHMB/MAA-g-PET fibers.

3.3. Thermal Stability Analysis of Original and Modified PET Materials

TGA is a thermal analysis technique in which mass changes are detected as a function of time and temperature (Bozkaya, 2023a; Bozkaya et al., 2022). TGA analysis was performed to determine the stability of the original PET fiber, MAA-g-PET fiber, and PHMB/MAA-g-PET materials against temperature in inert (N_2) gas atmosphere and to reveal the chemical modification. TGA/DTG thermograms of the materials are given in Figure 6. When the TGA/DTG curves of the original PET are examined, it is seen that it exhibits a one-step degradation behavior between 350 °C and 550 °C and the maximum degradation temperature (T_{max}) is 424 °C (Figure 6a). During the degradation process where mainly H_2O, CO, CO_2 and CH_4 gases were released, approximately 86% mass loss (14% residue) occurred at 600 °C. The results are consistent with the TGA data of PET in the literature (Gün Gök et al., 2021). When the TGA/DTG curves of MAA grafted PET fiber were examined (Figure 6b), two-step mass changes occurred in the temperature range of 30-400 °C with maximum temperatures (T_{max}) of 56 °C and 240 °C, respectively. The first step of these mass changes was attributed to moisture and/or solvent evaporation and the second step to chemical degradation of MAA polymer. The mass change at 443 °C is the characteristic degradation step of PET. At 600 °C, about 93.3% mass loss occurred (6.7% residue). In the TGA/DTG spinning of MAA-g-PET fiber modified with PHMB (Figure 6c), it is seen that mass loss occurs at T_{max} of 340 °C, which is different from the MAA-g-PET fiber mass loss steps. This step is attributed to the loss of guanidine and chloride from the broken biguanide groups by cleavage of the main chain structure of PHMB (De Paula et al., 2011). At the end of 600 °C, approximately 15.53% remained undegraded. Thus, TGA/DTG results confirmed the synthesis of PHMB/MAA-g-PET fiber. In addition, Figure 6d, where all thermograms are overlaid, shows that the thermal stability after modification is relatively decreased compared to the original PET fiber.

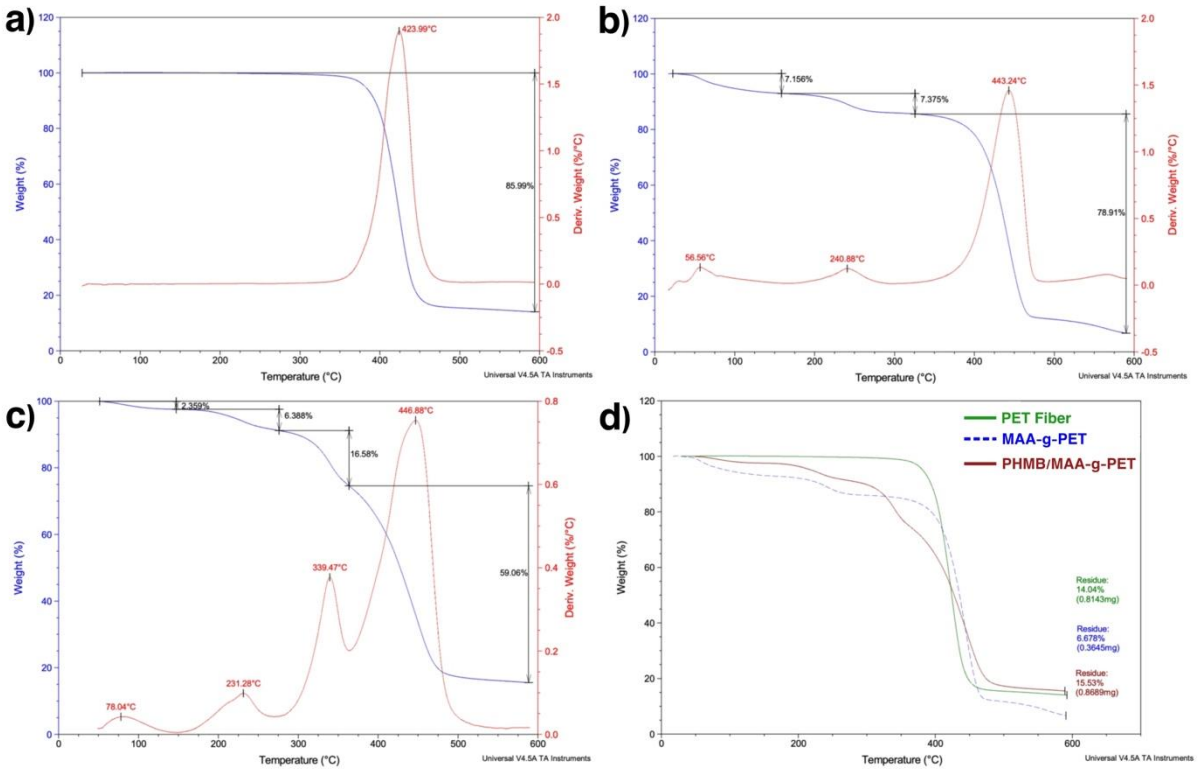


Figure 6. TGA/DTG thermograms of original **(a)** PET fiber, **(b)** MAA-g-PET and **(c)** PHMB/MAA-g-PET fiber, **(d)** overlaid TGA curves.

3.4. Characterization of Thermophysical Properties of Original and Modified PET Materials

DSC analysis was performed to determine the thermophysical properties such as crystallization temperature (Tc), glass transition temperature (Tg) and melting point (Tm) of the original PET fiber, MAA-g-PET fiber, and PHMB/MAA-g-PET fiber and the thermogram of the results are given in Figure 7. When the thermograms are examined, the Tg, Tc and Tm temperatures of PET fiber are approximately 75 °C, 105 °C and 253 °C, respectively. It is seen that the crystallization transition disappears in MAA grafted PET fiber. In addition, a broad endothermic transition between 180 °C and 250 °C occurred, involving the degradation of MAA, and melting of PET. In the DSC thermogram of MAA-g-PET fiber modified with PHMB, the endothermic transitions at 221, 248 and 339 °C are the temperatures of degradation of MAA, melting of PET and degradation of PHMB, respectively. The results agree with TGA, and it is proved that PHMB/MAA-g-PET fiber was synthesized.

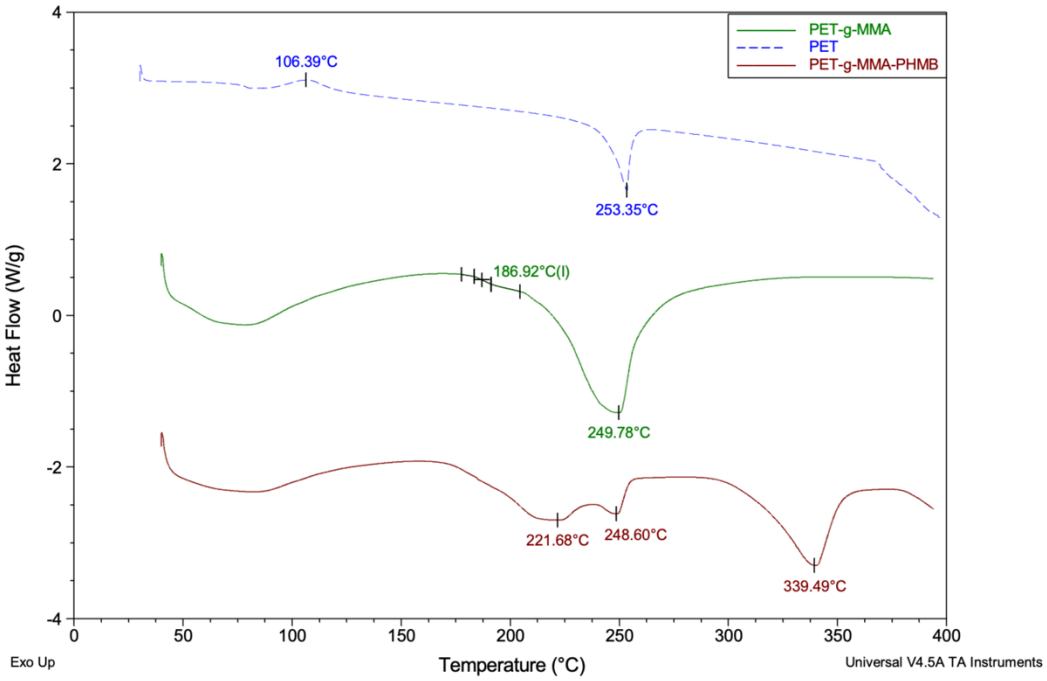


Figure 7. DSC thermograms of original PET, PET-g-MAA, and PHMB/MAA-g-PET fibers.

3.5. Wettability of PHMB/MAA-g-PET material

The water contact angle is an objective measure of a material's ability to either attract or repel water (Bozkaya, 2023b). Additionally, it serves as an indication of the self-cleaning characteristic of yarns (Liang et al., 2019). Materials with a water contact angle below 90º are categorized as hydrophilic, whilst those with a contact angle over 90º are classified as hydrophobic (Bozkaya et al., 2022). The contact angle is a characteristic that is influenced by the surface morphology, surface chemistry, and surface attributes (such as porosity, roughness, and surface energy) of materials (Chau et al., 2009). The variation of water contact angles of original PET fiber, MAA-g-PET, and PHMB/MAA-g-PET fiber is given in Figure 6. While the contact angle of the original PET fiber at the end of 30 s was 126º (Figure 8a) on average in accordance with its hydrophobic nature, the water contact angle of MAA-g-PET fiber was determined as 82º on average (Figure 8b). This can be attributed to the polar functional groups present in the structure of MAA. The contact angle of MAA-g-PET fiber modified with PHMB (Figure 8c) decreased to approximately 52º and its hydrophilicity increased. This may be due to the hydrophilic amine groups in the structure of PHMB. Wettability of surgical sutures is an important factor in surgical applications because adequate wetting of the suture line is important for proper placement of the suture and to promote healing. Good wettability allows the suture to pass more easily into the tissue and form a harmonious bond with the tissue. In addition, insufficient wetting of the suture line can cause the suture to slip into place or strain the suture line, which can lead to tissue trauma or weakening of the suture line (Anushya et al., 2022). Therefore, the wettability of PHMB/MAA-g-PET material supports its use as a surgical suture.

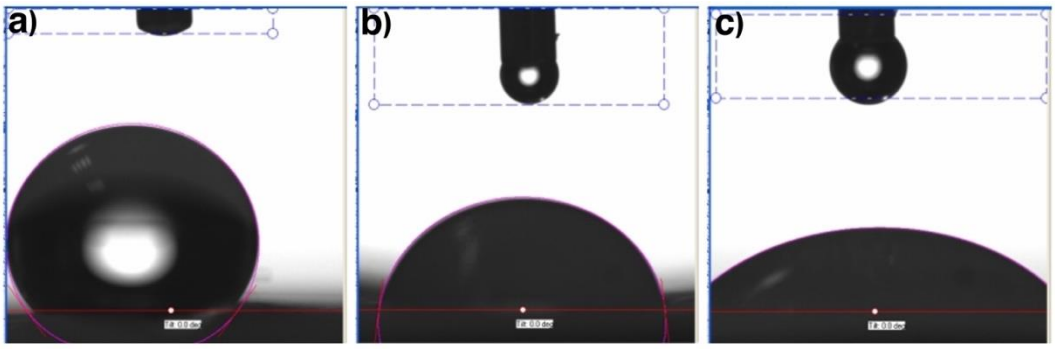


Figure 8. Water contact angle images of (a) original PET fiber, (b) MAA-g-PET fiber and (c) PHMB/MAA-g-PET fibers.

3.6. In vitro Degradation Profile of PHMB/MAA-g-PET Material

In this study, the in vitro degradation profile of the original PET and the PHMB/MAA-g-PET fiber synthesized as an antimicrobial surgical suture was examined. At the end of 7 days, approximately 1.21% and 1.25% mass loss occurred, respectively (Figure 9). It

was observed that the PHMB/MAA-g-PET fiber did not exhibit a significant degradation after grafting. Therefore, this reveals that the synthesized material retains its non-absorbable nature. PET sutures are non-absorbable materials preferred by many orthopedic surgeons for ligament or tendon repair. They are also used in body contouring surgery to fold the abdominal wall or to close abdominoplasty incisions. The surface of the PET suture can be coated with agents such as polybutylate to facilitate tissue passage and to make a smooth and strong knot. The inert nature of PET polymer minimizes the possibility of foreign body reaction. Therefore, it is very important that materials designed as non-absorbable sutures, but which are given antimicrobial properties with various agents, do not change the degradation profile and do not release components that may cause foreign body reactions (Byrne & Aly, 2019). The fact that the PHMB/MAA-g-PET material synthesized in this study does not exhibit a significant degradation behavior compared to the original PET is very promising in terms of in situ antimicrobial activity.

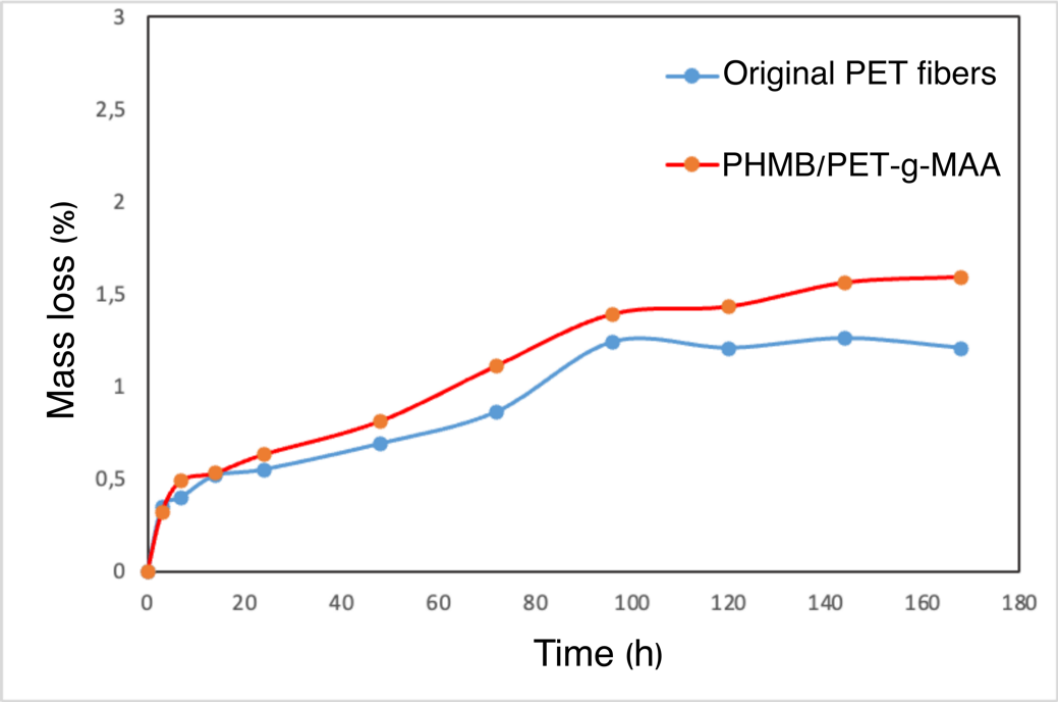


Figure 9. Degradation profiles of original PET and PHMB/MAA-g-PET fiber in PPS at 37 °C.

3.7. Antimicrobial Activity of PHMB/MAA-g-PET Material

In order to assess the antibacterial effectiveness of the synthesized PHMB/MAA-g-PET material, a minimum inhibitory concentration (MIC) experiment was conducted. The findings of this assay are presented in Figure 10. The PHMB/MAA-g-PET was found to have an almost 100% lethal effect on the bacterial species *S. aureus* and *E. coli*. Furthermore, it was seen that there was no proliferation in the wells maintained under sterile conditions and containing antibiotics, but the bacteria cultured in a sterile medium thrived on agar. Therefore, PHMB/MAA-g-PET material was proven to have antibacterial effect on both gram-positive and gram-negative bacteria. Like our findings, antibacterial activity of PHMB containing materials on *E. coli* and *S. aureus* species has been reported in the literature (Allen et al., 2006; Ashraf et al., 2012; Dilamian et al., 2013). PHMB is an agent that has been used as antiseptic and disinfectant in medicine, textile, and food industry for decades with proven antimicrobial activity against many bacterial and fungal species. PHMB is a positively charged polymer with biguanide units (Dilamian et al., 2013). PHMB shows bacteriostatic effect at concentrations lower than 10 µg/mL. At concentrations higher than 10 µg/mL, it shows bactericidal effect, while the potential for toxic effects increases (Ashraf et al., 2012; Gilbert et al., 1990). PHMB antibacterial activity is attributed to charge neutralization on the surface of the bacterial cell (Pal et al., 2007).

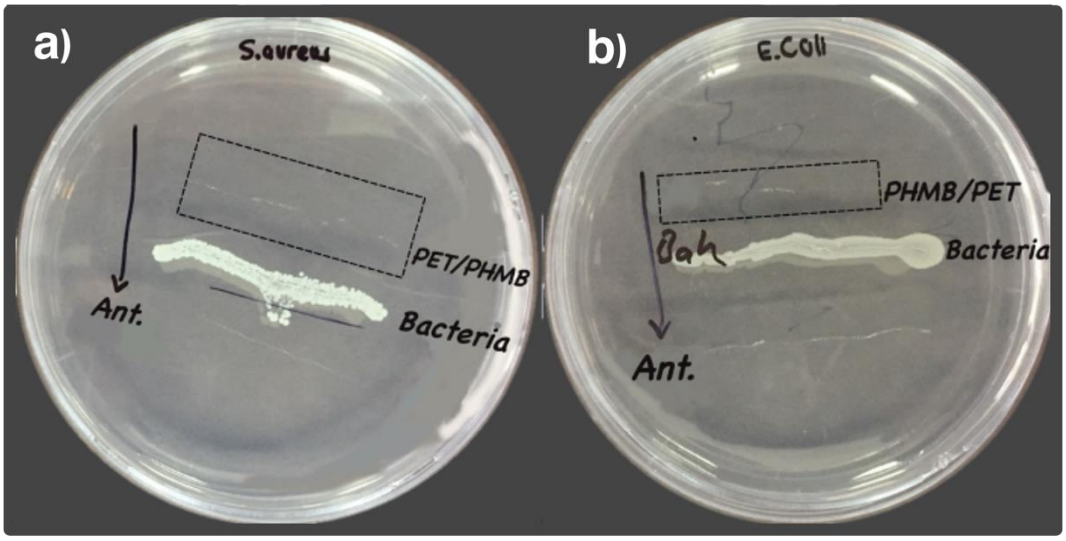


Figure 10. Petri images showing the antimicrobial activity of PHMB/MAA-g-PET material against **(a)** *S. aureus* and **(b)** *E. coli* bacteria species.

3.8. Assessment of In Vitro Cytocompatibility of PHMB/MAA-g-PET Material

The % viability values determined after 24 h incubation of PET fiber and PHMB/MAA-g-PET fiber extracts with L929 fibroblast cells were calculated compared to the control group and the change in cell viability with concentration is given in Figure 11. Cell viability was calculated as 82.60±4.34 at the highest concentration of 100% in the cells to which PET fiber was applied, while cell viability was 88.22±1.03 at 50% concentration. It was determined that cell viability increased as the applied concentration decreased. In the literature, the biocompatibility of PET fiber was investigated by MTT test in the study by Jiang et al. It was reported that there was no toxicity in 3T3 fibroblast cells treated with PET fiber and there was no significant difference in cell viability compared to the control group. In fibroblast cells treated with PHMB/MAA-g-PET fiber, cell viability was 69.88 ± 4.43 at 100% concentration and 75.42 ± 1.95 at 50% concentration. According to the evaluation criteria of TS EN ISO 10993-5 standard, if the cell viability is below 70%, it is stated that the material has cytotoxic potential. Therefore, based on the in vitro cytotoxicity test, it was concluded that the material produced in this study is cytocompatible in this respect and may be suitable for use as a surgical suture.

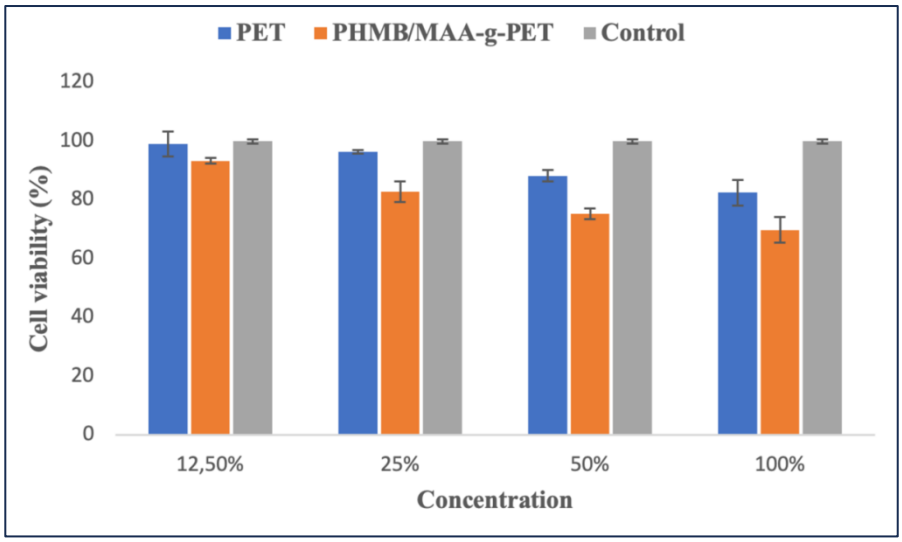


Figure 11. % cell viability values determined after 24 h incubation of original PET and PHMB/MAA-g-PET materials with L929 fibroblast cells.

4. Conclusions

The aim of this study was to synthesize a non-absorbable antimicrobial suture to prevent surgical suture-induced surgical site infections. For this purpose, firstly, MAA was successfully grafted to the multifilament PET fiber to provide a functional group to which PHMB could bind. MAA grafted PET fibers were treated with PHMB under certain conditions and immobilization of PHMB on PET fiber was achieved through carboxyl groups of MAA and positive amine groups of PHMB. The synthesized PHMB/MAA-g-PET material was characterized by techniques such as SEM, FTIR, TGA, DSC and all analyses revealed that the material was

successfully synthesized. In addition, water contact angle analyses showed that the synthesized material was wettable. In vitro degradation experiment was carried out to investigate whether the material retains its non-absorbable property and the results showed that PHMB/MAA-g-PET decreased in mass with a very small difference compared to the original PET and retained its non-absorbable property relatively. Antimicrobial tests on E. coli and S. aureus bacterial species proved that PHMB/MAA-g-PET was bactericidal against both species. In vitro cytocompatibility studies showed that the synthesized PHMB/MAA-g-PET material was not cytotoxic according to TS EN ISO 10993-5. In conclusion, the fact that the PHMB/MAA-g-PET material is not cytotoxic at the dose at which it shows the highest antimicrobial activity is very promising for surgical operations where non-absorbable sutures are required as surgical threads. However, extensive in vitro and in vivo experiments are required to make it a safe medical device.

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