



A comparative study of the physiochemical and biological properties of tetracycline-loaded polypropylene sutures prepared through different plasma treatments

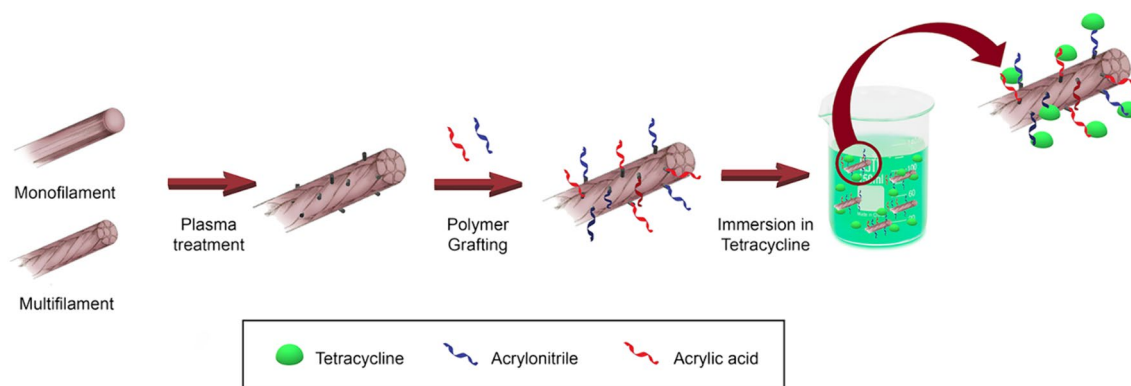
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Abstract

In this work, a comparative investigation was done on the physiochemical and biological properties of tetracycline-loaded polypropylene sutures prepared through nitrogen, oxygen, and/or argon plasma treatment/grafting method. Two types of multi- and monofilament polypropylene sutures were treated by various plasma gases followed by acrylonitrile and acrylic acid grafting. Nitrogen and oxygen plasmas compared to argon plasma showed higher efficiencies in functionalization and wettability of the filaments. Tetracycline hydrochloride loading on the plasma-treated/grafted filaments was done through immersion method. The prepared filaments were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and attenuated total reflection–Fourier transform infrared spectroscopy (ATR-FTIR) and their mechanical properties were determined, accordingly. The antibacterial effect of tetracycline hydrochloride was studied over two different Gram-positive and Gram-negative bacteria. No cytotoxicity was observed for these filaments on the L929 fibroblast cell line using MTT assay. The release profile of the tetracycline hydrochloride-loaded sutures reached the stationary phase in 25 h. Nitrogen plasma-treated sutures showed the highest drug release profile among the others. The current work showed the effects of different plasma treatments on the biological, mechanical, and chemical properties of the tetracycline-loaded polypropylene sutures. The nitrogen plasma was superior to oxygen plasma in the opinion of grafting rate, mechanical properties, and antibacterial activities.

Graphical abstract



Keywords Sutures · Plasma treatment · Tetracycline hydrochloride · Acrylonitrile · Acrylic acid

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Extended author information available on the last page of the article

Introduction

Surgical site infections are common nosocomial infections [1, 2]. These infections account for at least 20% of all hospital-acquired infections in the United States [3]. They are associated with an increased risk of mortality and the costs which are due to the increased length of stay, more frequent admissions to emergency departments and readmission [4].

To address this problem, wound closure methods have been considered as an area that needs improvement [5]. The sutures loaded with antibacterial agents have been vastly investigated [4, 6, 7]. Surface changing of sutures not only increased the biocompatibility, but also reduced bacterial colonization and infections at the scar site [8]. Different antimicrobial agents such as tetracycline hydrochloride (TC) [9], vancomycin [10], silver nanoparticles (AgNPs) [11], and ciprofloxacin [12] have been loaded on a variety of sutures. Various surface modification methods such as plasma [13, 14], irradiation [10, 15], immersion [16], and radiation methods [17] have been used to modify the surfaces of the sutures.

Plasma modification of the polymers is a common method for loading the desired drugs by creating functional groups on the polymers surfaces and increasing their surface reactivity [18, 19]. It has been generally shown that plasma treatments alter biological response and enhance biomaterial coating quality at the surfaces of the polymeric materials [20–22]. Plasma is often thought to alter the surface chemistry on only the outermost few nanometers of the material surface without changing its bulk properties [23, 24]. The plasma treatment creates a mixture of different kinds of functional groups on the surface.

When defined surfaces with homogenous functionalities are needed, grafting of specific monomers on the plasma-activated surfaces is recommended [25–28]. Polymerization of one or more functional monomers, e.g., vinyl imidazole [29], poly(*N*-vinyl-2-pyrrolidone) [30], and acrylic acid [26, 27] on the sutures surface has been done. The grafting of monomers onto the surfaces of polymers creates electrostatic charges and companion interactions, as well as hydrophilicity. Different methods have been studied for polymer grafting of PP filaments [27, 31].

Radiation grafting of acrylic acid monomers on the PP sutures followed by antibiotic loading has been reported for development of antibacterial sutures [10, 32]. Since PP can be cross-linked by radiation, the grafting radiation intensity must remain lower than PP threshold [15]. Saxena et al. have developed antibacterial PP sutures by plasma-induced graft polymerization of acrylic acid followed by chitosan binding on the emerged surface functional groups [31].

In addition to surface activation, plasma treatment of the sutures has been shown to prevent bacterial

colonization on the sutures, effectively, without altering their mechanical properties [22, 31, 33]. It was also shown by Learn et al. that uniformity and adherence of the polymerized sutures were increased after nonthermal plasma treatment [34].

In the current research work, a monofilament (commercial) and a multifilament (fabricated by melt spinning) polypropylene sutures were functionalized by different plasma treatments (oxygen, nitrogen and argon) followed by grafting with acrylonitrile and acrylic acid to increase their wettability and receptivity for TC. Afterward, TC was loaded on the sutures' plasma-activated/grafted filaments. The effects of different plasma treatments were investigated on the mechanical properties, biocompatibility, antibacterial effect, and drug release profile of the prepared sutures.

Experimental

Materials

Polypropylene suture (number zero) was purchased from Ethicon Incorporation (USA). PP granule ($\text{CH}_2\text{-CH}(\text{CH}_3)_n$) was purchased from Navid Zar Chimi (Iran). Acrylonitrile ($\text{C}_3\text{H}_3\text{N}$), acrylic acid monomers ($\text{C}_3\text{H}_4\text{O}_2$), potassium per sulfate ($\text{K}_2\text{S}_2\text{O}_8$), and methanol (CH_3OH) were purchased from Merck (Germany). Tetracycline hydrochloride (TC) ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\cdot\text{HCl}$) was purchased from Razak Pharmaceutical Co. (Iran).

Polypropylene suture melt spinning

Polypropylene filaments were melt spun by a pilot melt spinning unit (Automatik Co., Germany). The speed of the collector part (SW46, made by Barmag) was in the range of 1600–6000 m per min.

Plasma treatment on the filaments

Plasma treatments were done by hanging the filament inside a cylindrical plasma chamber with the dimension of 38 cm (diameter) \times 40 cm (height) (Fig. S1 in Supplementary file). Nitrogen, oxygen, and argon with 99.99% purity and flow rate of 20 SCCM were applied. The plasma power and the power supply frequency were 100 W and 56.13 MHz, respectively.

Optical emission spectroscopy (OES) was used for determination of the reactive species in plasma. Optical emission spectrometer (Avantes, Avaspec 3638-USB2) was used with 300 lines per mm diffraction light and 10 μm diffraction grating.

The contact angles of the plasma-treated polymers were measured using a contact angle goniometer device (Jikan

CAG-20, Iran) and the images were analyzed by Image J software.

Polymer grafting and drug loading

The plasma-treated PP filaments were grafted with acrylonitrile and acrylic acid (2:1 v/v) in glass vessels containing the monomers (30%) in methanol/water as the solvent based on the method by Taghizadeh and Mafakhery [35]. Potassium per sulfate (0.05% w/v) was used as the catalyst for polymerization at different temperatures (37–67 °C). After grafting, filaments were washed with deionized water for 2 h [35]. The sutures were then dried at 40 ± 2 °C for 30 min and the grafting percentage was calculated using the following formula:

$$\text{Grafting percentage} = \frac{(\text{filament weight after grafting} - \text{filament weight before grafting}) / \text{filament weight before grafting}}{\times 100}$$

After grafting, the filaments were soaked in sodium hydroxide (4%) for 4 h and then immersed in TC solution (2.5%) for 24 h at ambient condition.

Characterization of the sutures

The attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy was used in the range of 500–4000 cm^{-1} for studying the presence of functional groups on the filaments surface.

XRD analysis was performed on both TC-free and TC-loaded sutures (Philips, X'Pert MPD). SEM micrographs (Tescan Co., The Czech Republic) were prepared to study the morphology, shape, and surface changes of the sutures. The tensile properties of the sutures were investigated using a tensile tester (Universal Testing Machine—Servo Electrical Tensile 5 KN, Santam Co.) machine with the tensile speed of 50 mm/min. To monitor the TC release, 5 cm of each suture sample was rinsed with water and then immersed in 10 mL of phosphate buffer saline (PBS) solution and incubated at 37 °C. At different time intervals, 1 mL of the PBS was collected and the same volume of PBS was replaced. The TC content of the collected samples was measured by absorbance measurements at 274 nm using a UV–Vis spectrophotometer.

The antibacterial activity of the sutures was investigated against *Escherichia coli* and *Staphylococcus aureus*. The bacteria were cultured separately on the Luria–Bertani agar plates. The sutures were cut into 10 ± 2 mm pieces and placed on the surface of the LB agar. The zones of growth inhibitions were measured after overnight incubation of the cultures at 37 °C.

Cytotoxicity assessment

The sutures' extracts were prepared by placing 1 cm^2 of each suture in 200 μL of PBS and incubation at 37 °C under agitation (50 rpm). The L929 fibroblast cell line purchased from the Iranian Biological Resource Center cultured under standard cell culture condition and was treated with the of sutures extract. For cytotoxicity assessments, the cells were cultured in 96-well plates (10,000 cells per well) containing DMEM supplemented with 10% FBS for 24 h. Then, the culture media were replaced with DMEM (without FBS) and 20 μL of the suture extract was added to each well. After 24 h of incubation, 10 μL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Then, the culture media was removed and 100 μL DMSO was added to each well. After 15 min of gentle shaking, the absorbance of each well was measured with a microplate reader (BioTek, power wave Sx2). Each sample was tested in triplicate.

Results and discussion

Optimization of plasma treatment conditions

Plasma-derived reactive species are mainly classified into reactive oxygen species (ROS) and reactive nitrogen species (RNS) which have great effects on the biological properties of the plasma-treated materials. Applying high energy or electrical fields to the plasma gas chamber leads to gas ionization and formation of the reactive species [36].

The elemental composition of the plasma gases was investigated by OES analysis (Fig. S1 in Supplementary file). The main peaks in nitrogen plasma chamber were at 337, 359, and 391 nm which belong to the N_2^+ , N_3 , and N_2 species, respectively. In oxygen plasma, the amount of atomic oxygen plays a key role in the efficiency of the surface modification. O^+ , O^{2+} , O^{4+} , O^{3-} , O^{2-} , and O^- are different species generated in the plasma chamber [37]. The O_2^+ has a major role in the surface modification since other species are not stable and have very short lifetimes. Argon plasma contains different species including Ar^+ (positive ion), Ar^m (metastable argon atoms), Ar^f (resonant argon atoms), and Ar^p (4p state atoms) [38].

Oxygen, nitrogen, and argon plasma treatments on the PP films resulted in increased hydrophilicity of the treated surfaces. Figure 1a–c shows the changes in the water contact angle upon different plasma treatments. Type of the plasma gas and time of exposure are the factors influencing the surface activation. Among them, type of the plasma gas is the most important one through creating different kinds of functional groups such as carboxyl, ether, peroxidase, and hydroxyl on the surface.

The contact angle of the untreated suture was 87° which decreased to 5° and 10° after oxygen and nitrogen plasma treatment, respectively. Since argon is a noble gas with lower polarity compared to oxygen and nitrogen, the contact angle of the treated surface was higher (14°) compared to other plasma gas treated surfaces. It is noteworthy that during the plasma treatment, some of the functional groups are eliminated by the reactive elements in the plasma after a long time of exposure. Plasma OES results have been shown in Fig. S2 in Supplementary file.

Optimization of grafting condition

The temperature is a factor affecting the efficiency of grafting on the filaments. Figure 1d–f shows the efficiency of the mono and multifilaments grafting under different plasma gas treatments in a range of temperatures. Figure 1d–f shows that all the sutures prepared at different plasma gas conditions had the highest grafting rate at about 55°C . Multifilament sutures have a higher surface to volume ratio, providing more surface for grafting. Argon plasma-treated

filaments showed significantly less grafting than the other plasma-treated filaments.

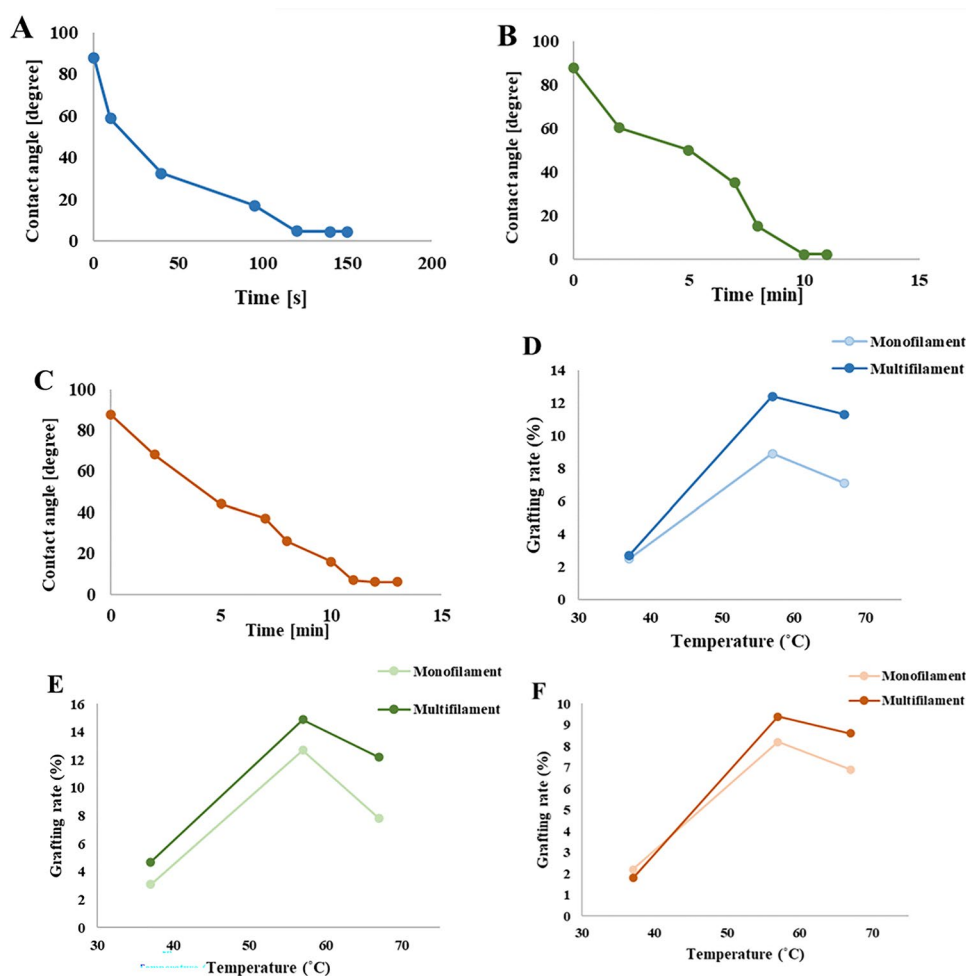
FTIR analysis of the grafted filaments

The ATR-FTIR analysis of the filaments showed notable changes in the functional groups after grafting. The most noticeable changes were the appearance of new peaks at the $1550\text{--}1750$, $2000\text{--}2200$, and $3000\text{--}3600\text{ cm}^{-1}$ regions which were attributed to C=O amide groups, C=C stretching, and O–H or N–H stretching vibrations, respectively (Fig. S3 in Supplementary file).

The nitrogen plasma-treated filaments showed absorption bands at 1630 cm^{-1} (C=C), 1752 cm^{-1} (C=O stretching), 1990 cm^{-1} (C=C), 2036 cm^{-1} (conjugated imine groups), and 2180 cm^{-1} (C≡N) [39, 40]. The wide peak at $3000\text{--}3500\text{ cm}^{-1}$ could be attributed to the hydroxyls.

In the oxygen plasma-treated filaments, the peak at $1000\text{--}1100\text{ cm}^{-1}$ corresponds to C–OH stretching vibration and the peak around 1760 cm^{-1} represents the stretching vibration of C=O groups. The peaks at $1380\text{--}1390\text{ cm}^{-1}$ and $1400\text{--}1500\text{ cm}^{-1}$ are attributed to CH_3 and CH_2

Fig. 1 Changes in the contact angle of the PP films upon different plasma treatments for different time periods: **a** oxygen, **b** nitrogen, and **c** argon plasma and effect of temperature on the grafting rate of mono- and multifilaments treated by **d** oxygen, **e** nitrogen, and **f** argon plasma



bending vibrations, respectively. In addition, the peak at 2900–3000 cm^{-1} shows the C–H stretching vibration (Fig. S3 in Supplementary file).

In the argon plasma-treated filaments, fewer surface functional groups were formed (Fig. S3 in Supplementary file). Thus, the oxygen and nitrogen plasmas were selected for further investigations.

Mechanical properties of the plasma-treated filaments

The knot and filament strength of the plasma-treated sutures were measured. As shown in Fig. 2a, b, the filaments had higher strength than the knots (122 MPa for monofilament and 71 MPa for the knot; 58 MPa for multifilament and 37 MPa for the knot) because of the accumulated tension pressure in knots compared to the filaments. Monofilament sutures showed higher strength (122 MPa) compared to the multifilament ones (58 MPa) (Fig. 2a). After oxygen plasma treatment, the strength reduction from 122 to 105 MPa in monofilament sutures and from 58 to 53 MPa in the multifilament ones were seen (Fig. 2c), while nitrogen plasma treatment had no effect on mono- and multifilament sutures strengths (Fig. 2d).

The values of elongation-at-break (fracture strain) in Fig. 2a, b showed that filaments had higher elongation-at-break values in comparison to the knots (71% for monofilament and 29% for knot; 37% for multifilament and 22% for knot). Generally, monofilament sutures (71%) showed higher

elongation-at-break values rather than the multifilament (37%). The elongation-at-break reduced to 44% in the monofilament and 28% in the multifilament sutures under oxygen plasma treatment (Fig. 2c), while these values remained without changes in the nitrogen plasma treatment (Fig. 2d).

The SEM micrographs of the plasma-treated/grafted multifilament and the monofilament sutures have been presented in Fig. 3. As shown in Fig. 3a the untreated multifilament suture, Fig. 3b shows that oxygen plasma had general changes on the surface of the filaments while nitrogen plasma had greater effects (Fig. 3c). Treatment of the monofilament suture with oxygen (Fig. 3e) and nitrogen (Fig. 3f) plasma has led to surface changes which are clearly observable due to the highly smooth and glossy surface of the filament (Fig. 3d). Similar to the multifilament suture, the nitrogen plasma had a greater effect than oxygen plasma on the surface morphology. The morphologies of the drug-loaded multifilament and monofilament sutures have been compared in Fig. 3 g and h, respectively.

TC loading on the plasma-treated/grafted sutures

After grafting the sutures with acrylonitrile and acrylic acid, the prepared sutures were loaded with TC. Drug-loaded multifilament revealed two new peaks in the FTIR spectrum indicating the drug loading on the sutures. The C=O amide peaks ($\sim 1650 \text{ cm}^{-1}$) and the alcohol O–H stretching vibration ($\sim 3500 \text{ cm}^{-1}$) can be attributed to the ketone and hydroxyl functional groups of TC, respectively (Fig. 4A, B).

Fig. 2 The strength measurements of: **a** untreated monofilament and multifilament sutures, **b** knots of untreated monofilament and multifilament sutures, **c** oxygen plasma-treated monofilament and multifilament sutures, and **d** nitrogen plasma-treated monofilament and multifilament suture

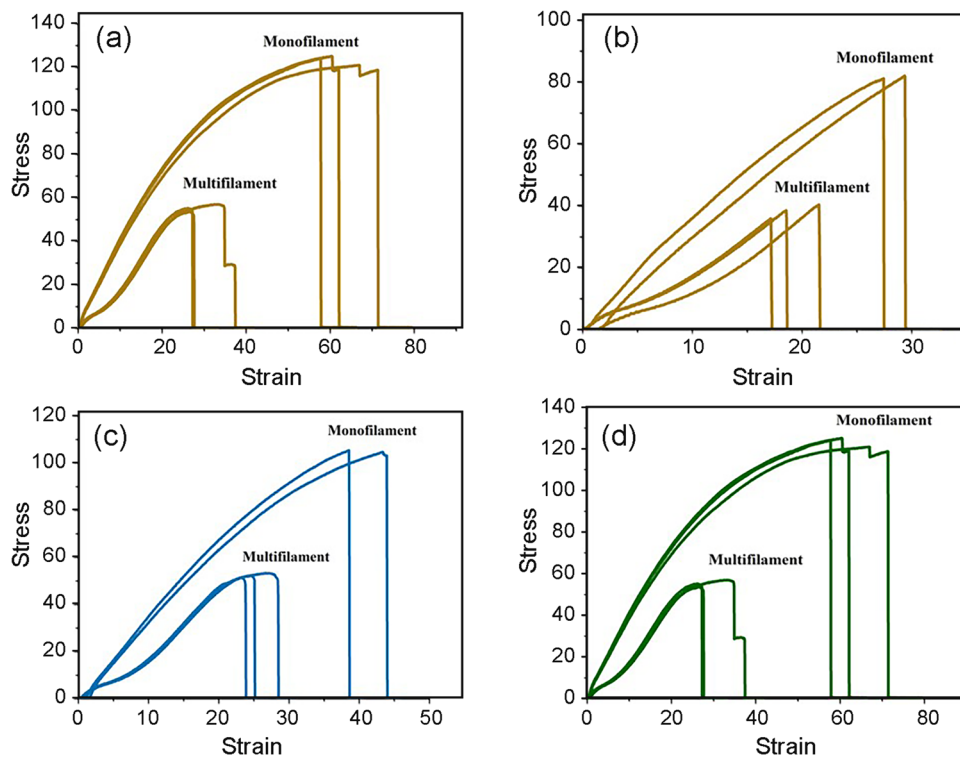
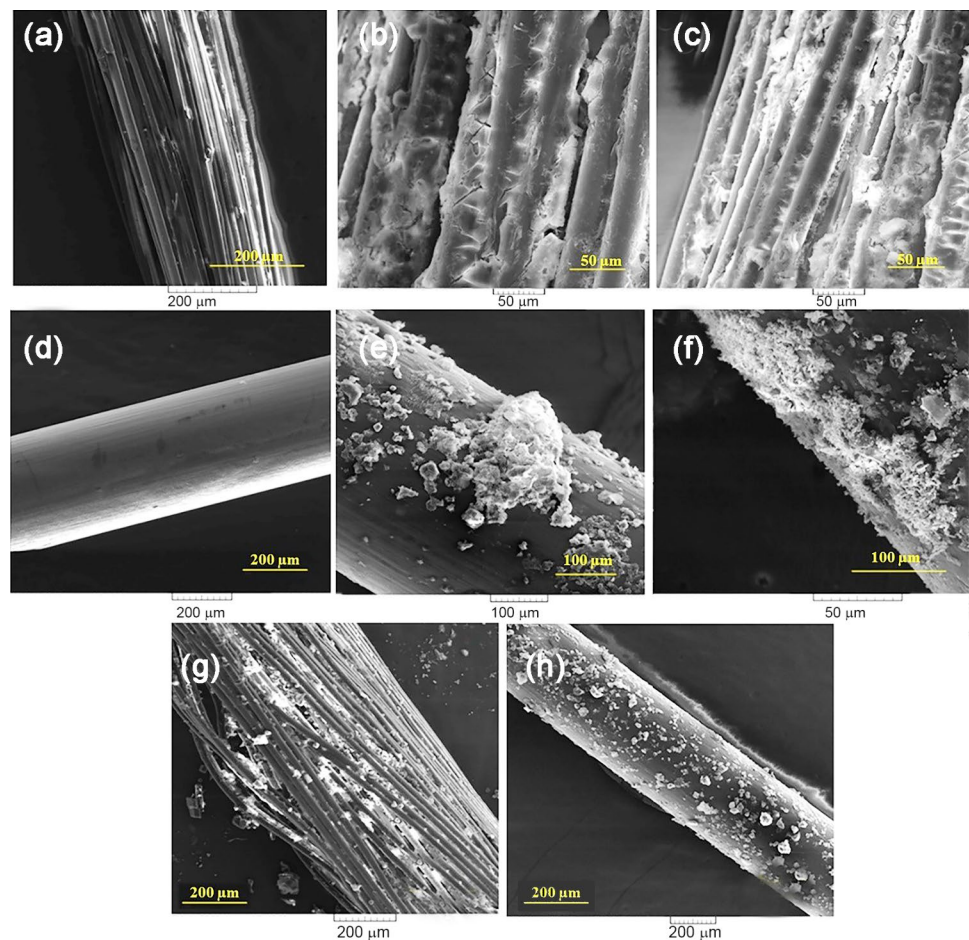


Fig. 3 SEM micrographs of: **a** untreated multifilament suture, **b** oxygen plasma-treated/grafted multifilament suture, **c** nitrogen plasma-treated/grafted multifilament suture, **d** untreated monofilament suture, **e** oxygen plasma-treated/grafted monofilament suture, **f** nitrogen plasma-treated/grafted monofilament suture, **g** multifilament nitrogen plasma-treated/grafted/drug-loaded sutures, and **h** monofilament nitrogen plasma-treated/grafted/drug-loaded sutures



XRD analysis (Fig. 4B) revealed no changes in the crystallinity after TC loading on the grafted sutures.

Drug release profile

The drug release profile reached the stationary phase after about 25 h in both the mono- and multifilament sutures as shown in Fig. 4C, D, respectively. Sutures grafted after nitrogen plasma treatment showed the highest drug release in both the mono- and multifilament sutures which was expected due to their higher grafting rate.

Antibacterial activity of the TC-loaded sutures

The antibacterial effects of the TC-loaded sutures were investigated on *Escherichia coli* and *Staphylococcus aureus* by the growth inhibition zone measurements (Fig. 4E). The inhibition zone was larger in sutures treated with nitrogen plasma in accordance with their higher grafting rate and drug release profile. On the other hand, the inhibition zone of the *Staphylococcus aureus* was larger than *Escherichia coli*, due to the inherent susceptibility of the Gram-positive bacteria to TC.

Biocompatibility assessment of the sutures

The cell viability assay was done on cells treated by the extracts of multifilament and monofilament sutures prepared at different plasma gas conditions (Fig. 4F). None of the sutures showed cytotoxicity. Viability differences were not significant under $p < 0.001$ tested by ANOVA using OriginPro 2022.

Comparative investigation on the physicochemical and biological properties of tetracycline-loaded polypropylene sutures prepared through nitrogen, oxygen, and argon plasma treatment/grafting showed the effect of type of plasma treatment. Other research works have been shown that plasma treatment alters the physical and chemical properties of the surface of biocompatible polymers without affecting their bulk properties. For example, Saxena et al. have developed antimicrobial PP sutures by plasma-induced graft polymerization of acrylic acid followed by chitosan binding on the remaining carboxyl groups. TC and silver nanoparticles were loaded as antimicrobial agents on these sutures. They showed that oxygen plasma treatment did not alter the physical and chemical properties of the PP sutures and had no adverse effect of their biological applications [31]. Gogoi

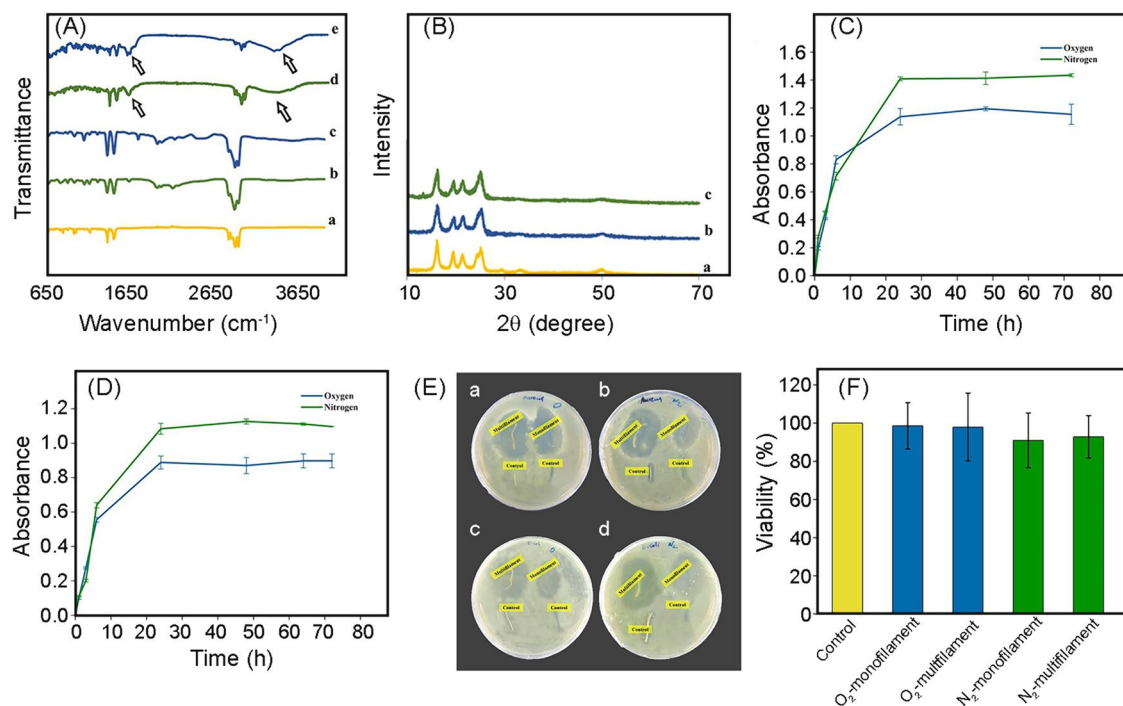


Fig. 4 **A** The ATR-FTIR spectra of (a) PP filament, (b) PP filament/nitrogen plasma treated/grafted, (c) PP filament/oxygen plasma treated/grafted, (d) PP filament/nitrogen plasma treated/grafted/TC loaded and (e) PP filament/oxygen plasma treated/grafted/TC loaded; **B** the XRD patterns of multifilament sutures: (a) PP filament, (b) PP filament/oxygen plasma treated, grafted/TC loaded, and (c) PP filament/nitrogen plasma treated/grafted/TC loaded; drug release profiles of **C** multifilament and **D** monofilament sutures prepared under dif-

ferent plasma gas conditions; **E** The zone of inhibition of: (a) oxygen plasma-treated sutures for *Staphylococcus aureus* (Gram positive), (b) nitrogen plasma-treated sutures for *Staphylococcus aureus*, (c) oxygen plasma-treated sutures for *Escherichia coli* (Gram negative), and (d) nitrogen plasma-treated sutures for *Escherichia coli*; **F** cytotoxicity of the multifilament and monofilament drug-loaded sutures prepared under different plasma gas conditions

et al. have studied the effect of PP grafted muga silk (*Antheraea assama*) using Ar-plasma treatment. This suture showed good mechanical, antibacterial, and wound healing properties [41]. Anjum et al. have functionalized poly (ethylene terephthalate) sutures with silver ions and Aloe Vera through carbon dioxide plasma treatment [7]. In another study by Anjum et al., carbon dioxide plasma-treated PET sutures were loaded with silver nanoparticles and chlorhexidine. The developed sutures had antibacterial effect on both Gram-positive and Gram-negative bacteria [42]. Serrano et al. showed that oxygen plasma treatment generates nanostructures at the surface of commercial sutures of different composition. The presence of nanosized topographies prevented bacterial attachment and biofilm formation without compromising physical properties and biocompatibility of the sutures [43]. Ercan et al. showed that nonthermal atmospheric plasma treatment substantially increased the hydrophilicity of sutures which might be the primary mechanism for the prevention of bacterial colonization [33]. The present work compared the effects of oxygen, nitrogen, and argon plasmas on the physicochemical and biological properties of PP sutures. The results showed that nitrogen plasma was superior to oxygen and argon plasmas in the opinion of grafting

rate, mechanical properties, and antibacterial activity on both Gram-positive and Gram-negative bacteria.

Conclusion

Plasma treatment as a surface modification strategy has been applied by researchers to functionalize the surface of the polymeric biomaterials including sutures. Herein, the effects of three different plasma gases (oxygen, nitrogen, and argon) were investigated on the physicochemical, mechanical, and biological properties of PP filaments. To the best of our knowledge, this is the first comparative study of the effects of different plasma treatments on the graft polymerization, drug loading and antibacterial activity of the PP sutures. It was shown that nitrogen plasma treatment was superior in the opinion of the grafting rate, resulting in the highest drug loading and antibacterial activity.

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Author contributions Kasiri N contributed to the design and implementation of the research. Mousazadeh M and Mousazadeh F were

responsible for writing the main manuscript and data analysis. Nikkhah M conceptualized this research and reviewed the manuscript. Keshvari H reviewed the whole manuscript.

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Data availability All the data related to this article will be available upon request from the authors.

Declarations

Conflict of interest The authors declared no conflict of interests.

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