Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 280 (2004) 173–183
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Antimicrobial activity of polyurethanes coated with antibiotics: a new approach to the realization of medical devices exempt from microbial colonization

A. Piozzi [a,*], I. Francolini [a], L. Occhiaperti [a], M. Venditti [b], W. Marconi [a]

[a] *Department of Chemistry, University "La Sapienza", P.le Aldo Moro, P.O. Box 32, Rome 00185, Italy*
[b] *Department of Clinical Medicine, University "La Sapienza", P.le Aldo Moro, P.O. Box 32, Rome 00185, Italy*

Received 3 December 2003; received in revised form 17 May 2004; accepted 20 May 2004

Abstract

Intravascular devices are widely used for vascular access but are associated with substantial risk of development of devices-related bloodstream infection (DR-BSI), which causes a considerable increase of morbidity and mortality, prolonged hospitalisation and growing medical costs. Since conventional treatment of DR-BSI fails in a significant number of cases, resulting in removal of the device, new approaches are needed to prevent bacterial colonization. In this paper, two antibiotics, rifampin and amoxicillin, have been adsorbed on polyurethanes exhibiting acidic or basic properties. The influence of the type of antibiotic–polymer interaction on the amount of adsorbed antibiotic and on the release kinetics was studied. It was seen that the antibiotic–polymer affinity increases both with the introduction in the polymer side-chain of functional groups and with the matrix hydrophilicity. The antimicrobial activity of the treated polymers, evaluated in vitro by the Kirby–Bauer test, depends on the amount of antibiotic adsorbed, on the strength of drug–matrix interaction and on the water swelling of the polymers. The inhibition zone of bacterial growth lasts only a few hours for the amoxi-coated polymers while remains at least for five months for the rifampin-coated ones. The presence of serum proteins decreases by about 30% the inhibition zone diameter of these latest matrices after two months.

Keywords: Central venous catheters; Polyurethanes; Rifampin; Amoxicillin; Antibiotic coating; Device-related infections

1. Introduction

Central venous catheters (CVCs) are used with increasing frequency in the intensive care unit (ICU) and in general medical wards. Catheter infection, the most frequent complication of CVC use, is associated with increased morbidity, mortality, and duration of hospitalization. Particular attention should be paid to the moment of their insertion in the patient and during all the time of implantation to reduce the risk of development of local or systemic infections (O'Grady et al., 2002; Donelli et al., 2001).

Risk factors in the development of catheter colonisation and bloodstream infection include patient factors (increased risk associated with malignancy, neutropenia, and shock) and treatment-related factors (increased risk associated with total parenteral nutrition, ICU admission for any reason, and endotracheal intubation). Other risk factors are prolonged catheter indwelling time, lack of asepsis during CVC insertion, and frequent manipulation of the catheter.

* Corresponding author. Tel.: +39 06 49913692; fax: +39 06 49913692.
E-mail address: antonella.piozzi@uniroma1.it (A. Piozzi).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.05.017

The pathogenesis of catheter-related infections is multifactorial and takes place through the following crucial steps: (i) colonization or contamination of the patient's skin with a resident or transient microbial opportunist; (ii) microbial migration through the insertion site into the cutaneous tract; (iii) colonization of the catheter tip by the potential pathogen.

The microrganisms colonize the device surfaces forming a slimy layer called "biofilm" that is believed responsible of the increased resistance to antibiotics of the consequent infections (Donlan and Costerton, 2002; Mah and O'Toole, 2001).

In intravascular catheter-associated infections, the most involved microrganisms are coagulase negative staphylococci (CNS), accounting for about 40%, *Staphylococcus aureus*, accounting for about 20%, and fungi, particularly *Candida* species, accounting for about 10% (Danese, 2002).

The recommended prevention strategies include: (i) educating and training of health care providers who insert and maintain catheters; (ii) using full barrier precautions during central venous catheter insertion; (iii) using a 2% chlorhexidine preparation for skin antisepsis; (iv) using antiseptic/antibiotic impregnated short-term central venous catheters.

The last method of prevention represents one of the most promising strategies developed during the last years (Elliott, 2000; Donelli and Francolini, 2001): in fact, the antimicrobial agent adsorbed on the catheters is released directly at the infection site without reaching systemic levels in the body. The good results obtained stimulated the study of new antibiotic–polymeric material associations aimed at improving the amount of adsorbed antibiotic and the general performance of the antimicrobial devices (Heard et al., 1998; Sheng et al., 2000; Kamal et al., 1998; Raad et al., 1998; Marconi et al., 2001; Marciante et al., 2003).

In this paper, two antibiotics, rifampin and amoxicillin, have been adsorbed on several polyurethanes to define the system that was more able to inhibit microbial colonization. The employed polyurethanes exhibited acidic or basic properties due to the different functional groups introduced in the polymer side-chain, and the antibiotics (active against gram-positive bacteria) possessed functional groups able to interact with the polymers tested.

The influence of the type of antibiotic–polymer interaction on the amount of antibiotic adsorbed and on the kinetics release was studied. The antimicrobial activity of the polyurethanes treated with antibiotics was assayed in vitro by optical microscopy and the Kirby–Bauer test.

2. Materials and methods

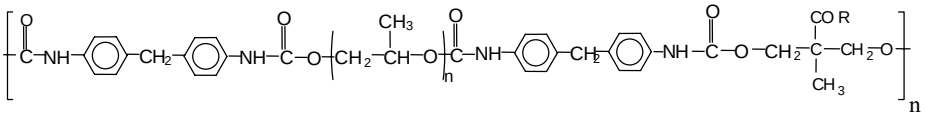
2.1. Materials

Methylen-bis-4,4 phenyl-isocyanate (MDI, Polyscience Inc.), 2-diethylamino-ethylamine (DED, Fluka) were distilled before use. Polypropylene oxide, mol. wt. 1118 (PPO, Fluka) was degassed, under vacuum, at 60 °C for 12 h. Tetrahydrofuran (THF, Fluka), dihydroxymethyl-propionic acid (DHMPA, Aldrich), *N*-hydroxy-succinimide (HSI, Fluka), dicyclohexylcarbodiimide (DCC, Fluka), Pirydine–SO_3 adduct ($PySO_3$, Carlo Erba), ethanolamine (EA, Fluka) and methyl iodine (CH_3I) were used as received. Rifampin (RIF) was purchased from Lepetit, Italia, while amoxicillin (AMOXI) and amoxicillin sodium salt (AMOXI*Na) were purchased from Ibi, Italia.

2.2. Polymer synthesis

A polymer provided with reactive functional groups (carboxy group) was obtained by a two-step condensation of MDI, PPO and DHMPA in the 2:1:1 ratio as previously reported (Marconi et al., 1996). The synthesised polymer was called PEUA (Fig. 1a).

In order to introduce basic groups in the polymer side chain, the carboxyl groups of PEUA were reacted with DED, obtaining the polymer called PEUADED (Fig. 1b). As for the amidation reaction, the carboxyl groups of PEUA were activated adding HSI and DCC, in equimolar amounts with respect to the –COOH groups, to a 5% (w/w) solution of PEUA in THF. The activation phase was carried out for 3 h, at 0 °C under stirring. The temperature was then increased to 25 °C and the amine DED was added in stechiometric ratio with the carboxy groups of the polymer. The reaction was carried out for 24 h at room temperature, under stirring. At the end of the reaction, the newly formed dicyclohexylurea was filtered; the polymer was precipitated in the aqueous solution and then recovered and dried under vacuum



a) PEUA R = -OH

b) PEUADED R = -NH-CH_2-CH_2-N(CH_2-CH_3)$_2$

c) PEUADEDQ R = -NH-CH_2-CH_2-N^+(CH_2-CH_3)$_2$(CH_3)

d) PEUEA R = - NH-CH_2-CH_2-OH

e) PEUEASO_3H R = -NH-CH_2-CH_2-O-SO_3H

Fig. 1. Repeating units of the polymers used for antibiotic adsorption.

at 30 °C for 3 days. The degree of amidation of PEUADED was determined by titration of the amino groups with 0.1 N HCl and bromophenol blue as indicator.

To obtain a polymer provided with positively charged groups, the tertiary amino groups of PEUADED were quaternized with CH_3I.

The quaternization reaction was performed adding CH_3I (5:1 stechiometric ratio CH_3I:tertiary amino groups of the polymer) to a 5% (w/w) solution of PEUADED in $CHCl_3$. The polymer obtained, PEUADEDQ (Fig. 1c), recovered by evaporation of the solvent and of the CH_3I excess under vacuum and at room temperature, was then dried under vacuum at 60 °C for 3 days. The yield of the reaction was determined by acidic titration of the residual unreacted amino groups.

Moreover, to obtain a polymer provided with marked acidic properties, –SO_3H groups were inserted in the PEUA side chain. To this aim, its carboxyl groups were amidated with EA, so introducing hydroxy groups that were subsequently reacted with $PySO_3$. The reaction was performed under the same conditions adopted for the amidation with DED. The polymer obtained was called PEUEA (Fig. 1d). The reaction with $PySO_3$ was conducted adding this reagent, at $PySO_3$:OH ratio of 2.5:1, to a 3% (w/v) solution of PEUEA in anhydrous DMF. The reaction was carried out for 1 hr at 0 °C and then 23 h at room temperature under dry nitrogen flow. The polymer (PEUEASO_3H, Fig. 1e), recovered by precipitation with dilute NaOH solution (pH = 8), was dried under vacuum at 30 °C for 3 days. The degree of sulfation was determined by ^1H NMR spectrometry and by elemental analysis.

2.3. Preparation and sterilization of polymeric films

The antibiotics were adsorbed on polymeric disks (films), of about 3 cm in diameter and 150 μm in thickness. The films were prepared by deposition, on teflon plates, of a 5% (w/v) THF polymer solution and by solvent evaporation in vacuum oven at 30 °C.

In order to sterilize the polymeric films before antibiotic adsorption they were inserted in a laminar flow hood and were radiated with UV-rays for 24 h. The absence of bacterial growth on the sterilized films was evaluated by optical microscopy, after incubation at 37 °C for 24 h, in a suitable culture media (Muller Hinton broth, OXOID).

2.4. Antibiotics adsorption and analytical determination

The adsorption of the antibiotics was performed by dipping the polymer films in to an antibiotic 2% (w/v) aqueous solution, corresponding to a concentration of 0.05 M for AMOXI and AMOXI*Na and of 0.02 M for RIF. This adsorption reaction was carried out for 24 h, at room temperature, under mild stirring. Thereafter, to eliminate the unadsorbed antibiotic, the films were washed twice for 1 h with saline solution.

The analytical determination of bonded antibiotic was effected by UV–vis spectroscopy, by difference between the antibiotic content of the solutions

used, before and after the impregnation (plus that of the washing solutions). The solutions were analysed at 270 and 490 nm, for AMOXI and RIF, respectively. The molar extinction coefficients were $1300\,M^{-1}\,cm^{-1}$ and $14{,}000\,M^{-1}\,cm^{-1}$.

2.5. In vitro release studies

The kinetics of the antibiotic release from the polymeric films were studied carrying out washings, under mild stirring, in a saline solution and evaluating the amount of the released antibiotic in the washing solutions by UV–vis spectroscopy. To better mimic the in vivo situation corresponding to a catheter in contact with a continuously renewing biological fluid, the washing solution was changed every hour for the first 5 and each 24 h for the longer times.

At regular elution times, the films were dried in vacuum oven at 30 °C and their antibacterial activity was checked. In particular, the ability of drug-loaded polymers to inhibit bacterial growth was evaluated beginning from 2 h of washing, then every hour up to 5 h and each 24 h for longer times (up to 10 months for RIF-loaded films).

In order to evaluate the influence of the serum proteins on the antimicrobial effect durability of the realized antibiotic–polymer systems, the films treated with antibiotics were dipped in to human serum and incubated at 37 °C for increasing times. In this case, washing solutions (5 ml serum sufficient to cover the films) were renewed every day up to two months. Consequent results were compared with those obtained by polymers treated with saline solution.

2.6. Water swelling properties of the polymers

The hydrophilicity of the polymers was evaluated by studying the kinetics of water swelling of the matrices, before and after the antibiotic treatment. The measurements were performed by dipping the polymer films into distilled water at room temperature for different times. The percentage of swelling (S_w) is given by the following equation:

$$S_w = \frac{S - S_0}{S_0} \times 100$$

where S_0 is the initial weight of the film and S the weight of the swollen film (dried by a smooth treatment with filter paper).

2.7. In vitro antimicrobial tests

The antibacterial activity of the antibiotic-treated films was assessed in vitro by a modified Kirby–Bauer test. The polymeric films were placed in petri plates containing Muller–Hinton agar (MH, OXOID) previously seeded with 10^8 cfu/ml (0.5 McFarland) of the *Staphylococcus epidermidis* (ATCC 35984 and ATCC 12228, methicillin-resistant and -sensitive strains, respectively) and *S. aureus* strains (methicillin-resistant and -sensitive clinical isolates). After incubation at 37 °C for 18 h, the size of the inhibition zone of the bacterial growth around the antibiotic coated films was measured.

When an inhibition zone was not detected, the possible bacterial growth on the polymeric surfaces was assessed by observation with optical microscopy. To this aim, the polymeric surfaces were streaked in a drop of a sterile saline solution on a glass slide. The staphylococci present on the slide were fixed, stained with gram's stain and then observed by an immersion optical microscope. The initial bacterial adhesion and the possible presence of biofilm onto no longer active polymer surfaces were also evaluated by scanning electron microscopy (SEM, LEO1450VP instrument). The polymer films first were washed with a sodium cacodilate solution (0.1 M) and then treated with a 2.5% (w/w) glutaraldehyde solution containing also sodium cacodilate. Successively, after three washing with cacodilate solution, the films were treated with a 1% (w/w) osmium tetroxide solution in sodium cacodilate and washed again with the sodium cacodilate. The samples were successively dehydrated in a graded series of ethanol, dried in a critical point drier 010 Balzer employing CO_2 as transition fluid, and finally shadowed with gold.

3. Results

3.1. Polymer synthesis

Under the adopted experimental conditions, PEUA contained a carboxy group per repeating unit, as

Table 1
Elemental analysis of PEUEASO$_3$H

Atomic percentage	C%	N%	H%	S%
Calculated[a]	42.4	2.5	6.04	1.14
Found	42.4	2.82	6.09	0.71

[a] The calculated atomic percentage corresponds to a degree of sulfation of 100%.

evidenced by titration and ^{1}H NMR measurements (Marconi et al., 1996).

The introduction of basic groups in the polymer side-chain of PEUA allowed to obtain a polyurethane, PEUADED, possessing 65% of tertiary amino groups, while the yield of the quaternization reaction of the amino groups of PEUADED was total, as evidenced by acidic titration. The amidation of the carboxy groups of PEUA with ethanolamine was complete.

The yield of the sulfation reaction, determined by elemental analysis (Table 1), was 62%. This result was confirmed by ^{1}H NMR measurements (Fig. 2). The degree of sulfation was determined by the ratio between the integral of the peak at 3.8 ppm, corresponding to the resonance of the methylene group of MDI, and that of the peak at 4.13 ppm, relevant to the methylene group near to the –SO$_3$H.

Table 2
Amount of the antibiotics adsorbed onto polyurethane films

POLYMER	Adsorbed amount of AMOXI[a] (mg/cm^2)	Adsorbed amount of RIF[a] (mg/cm^2)
PEUA	0.55 ± 0.02	NP[c]
PEUADED	0.73 ± 0.02	0.56 ± 0.05
PEUADEDQ	0.32 ± 0.05[b]	0.9 ± 0.1
PEUEA	0	0.088 ± 0.002
PEUEASO$_3$H	1.3 ± 0.2[b]	0.7 ± 0.1

[a] Residual drug bonded to polymer after 2 h washing time.
[b] Polymers treated with AMOXI*Na.
[c] NP: not performed.

3.2. Antibiotics adsorption and "in vitro" release studies

The amount of the two antibiotics adsorbed on the differently functionalysed polyurethane is reported in Table 2. As for RIF, it was not possible to perform its adsorption on PEUA since the polymer dissolves in its basic aqueous solution (pH = 9).

UV determination demonstrated that the drug release is present only during the first 5 h. Moreover, while for AMOXI the antibiotic elution from PEUADED and PEUA was about 40 and 45%, for AMOXI*Na, the release from PEUADEDQ and

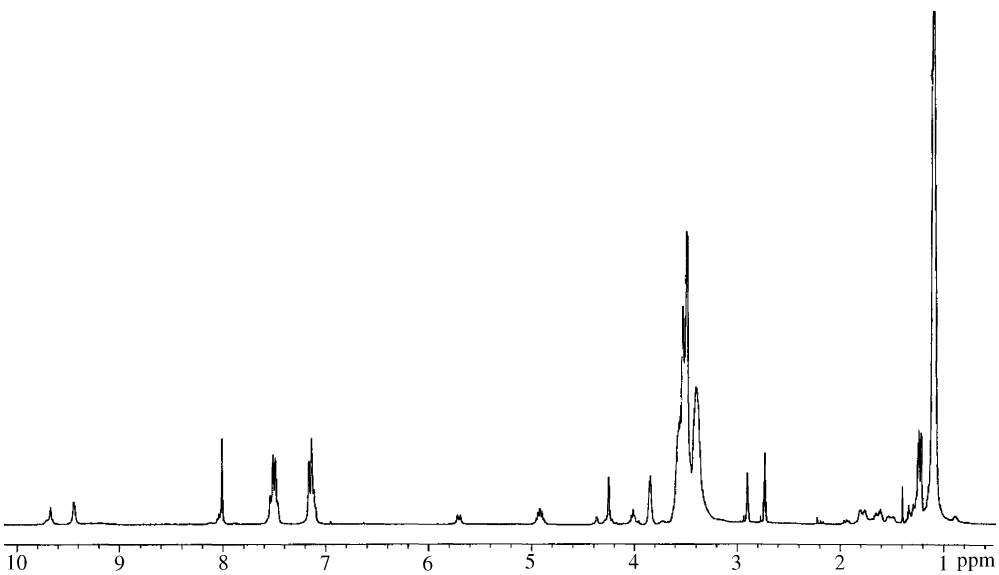


Fig. 2. ^{1}H NMR spectrum of PEUEASO$_3$H.

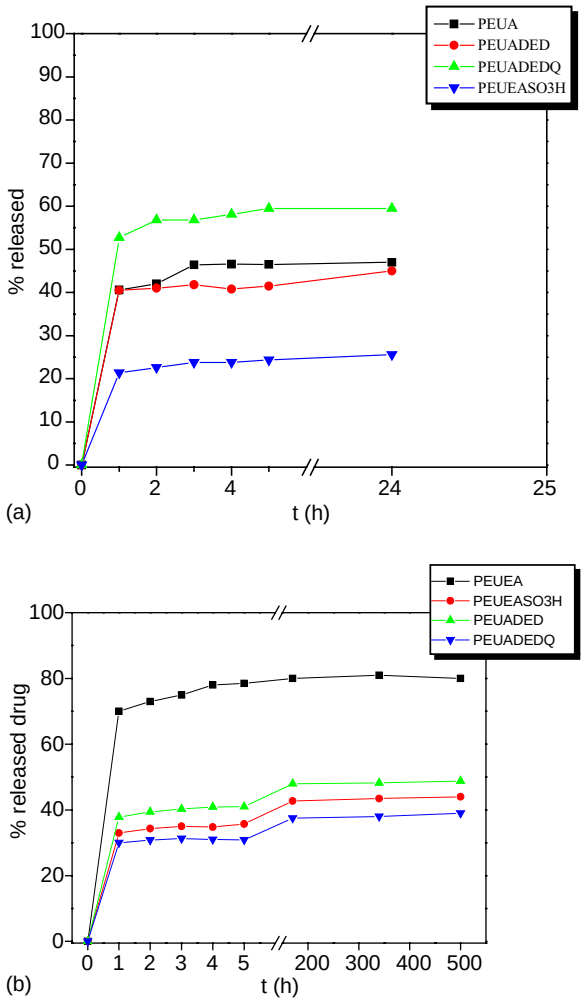


Fig. 3. Elution curves of AMOXI (a) and of RIF (b) from the differently functionalised polymers.

$PEUEASO_3H$ was about 60 and 25%, respectively (Fig. 3a).

In case of RIF, PEUADEDQ and $PEUEASO_3H$ on the contrary show a release value of about 30 and 35% respectively, compared to release values of about 40% for PEUADED and 78% for PEUEA (Fig. 3b).

3.3. Water swelling kinetics

In Fig. 4a the water swelling kinetic curves of the different polyurethanes, before antibiotics adsorption, are reported. The carboxyl groups contribute to hydrophilicity more than hydroxy (PEUA and PEUEA show after 120 h a swelling of 30 and 15%, respectively), and the introduction of tertiary amino groups increases the hydrophylicity of the polymer (PEUADED shows after 120 h a 40% swelling).

The swelling measurements were even carried out onto the rifampin–polymers after the antibiotic adsorption (Fig. 4b). The obtained data evidence that the drug adsorption decreases the swellability of the hydrophylic matrices. In fact, $PEUEASO_3H$ hydrophilicity was reduced about 60% (from 210 to 90%) while that of PEUADEDQ by about 80% (from 190 to 40%).

3.4. Antimicrobial activity of polymeric films

The antimicrobial activity of the antibiotic–polymer systems was assayed by the Kirby–Bauer test. The presence of an inhibition zone of the bacterial growth around the polymer films proves that the adsorbed antibiotics maintain their antimicrobial activity. In Tables 3 and 4, the maximum washing times after which the inhibition zone can still be observed are reported.

The polymers treated with AMOXI demonstrated a poor antibacterial activity both against the methicillin-sensitive (*S. epidermidis* ATCC 12228 and clinical *S. aureus*) and methicillin-resistant strains (*S. epidermidis* ATCC 35984). Indeed, the inhibition zones of the bacterial growth (Table 3) from a few hours up to any days were observed. In the case of methicillin-resistant *S. aureus*, as expected, polymers did not show any antibacterial activity. On the contrary, the films adsorbed with RIF are able to inhibit bacterial growth of the *S. epidermidis* up to several months of washing in saline solution (10 months for PEUADEDQ). In Table 4, the antimicrobial activity of treated-RIF polymers tested against the less virulent *S. epidermidis* are reported. A similar behaviour was also observed when polymers have been tested against *S. aureus* strain.

The data relevant to antimicrobial activity of the polymeric disks treated with rifampin and incubated in human serum for times varying from 24 h up to 2 months, compared with those relevant to the polymers treated with saline solution are reported in Table 5. A significant reduction (about 30%) in the inhibition zone diameter after 60 days of incubation in human serum was observed. When polymer surfaces do not show any inhibition zone the initial bacterial adhesion

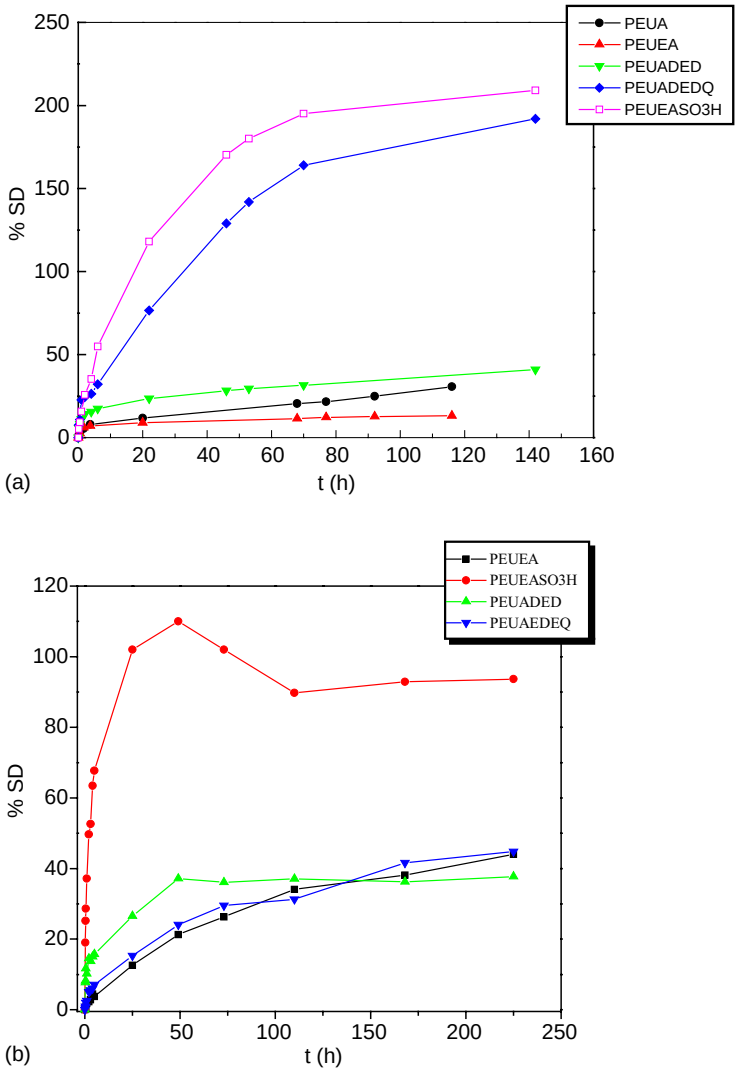


Fig. 4. Kinetics of the water swelling of polymers before (a) and after (b) RIF adsorption.

Table 3
Diameter of the inhibition zone measured around the treated-amoxi polymer films (beginning from 2 h of washing) and their antibacterial efficacy term

Polymer	AMOXI					
	S. epidermidis ATCC 35984		*S. epidermidis* ATCC 12228		*S. Aureus* methicillin-sensitive	
	Diameter of inhibition zone (mm)	Antibacterial efficacy term (h)	Diameter of inhibition zone (mm)	Antibacterial efficacy term (h)	Diameter of inhibition zone (mm)	Antibacterial efficacy term (h)
PEUA	4 ± 1	2	0	2	16 ± 1	24
PEUADED	5 ± 1	5	6 ± 1	24	14 ± 1	24
PEUADEDQ	6 ± 1	24	2 ± 1	48	21 ± 1	72
PEUEA	0	0	0	0	0	0
$PEUEASO_3H$	3 ± 1	2	0	2	11 ± 1	5

Table 4
Diameter of the inhibition zone measured around the treated-rifampin polymer films beginning from 2 h of washing and their antibacterial efficacy term

POLYMER	RIFAMPIN	
	S. epidermidis ATCC 35984	
	Diameter of the inhibition zone (mm)	Antibacterial efficacy term (months)
PEUA	NP[a]	NP[a]
PEUADED	32 ± 1	8
PEUADEDQ	35 ± 1	10
PEUEA	30 ± 1	4.5
$PEUEASO_3H$	32 ± 1	5

[a] NP: not performed.

and the possible presence of biofilm were evaluated by scanning electron microscopy. In Fig. 5 as example, SEM micrographs of AMOXI loaded PEUADEDQ with and without bacteria (ATCC 12228 *S. epidermidis* strain) are reported. It can be observed that the polymer–antibiotic system is able to express antibacterial activity for 48 h (Fig. 5a), according to the observation with optical microscopy. At 72 h the polymer showed a pronounced bacterial adhesion and finally at 96 h the presence of biofilm and microclusters of bacterial growth is evidenced (Fig. 5b and c respectively).

4. Discussion

The present research aimed at the development of antimicrobial–polymer models able to prevent infections associated with the use of medical devices. In the preparation of antibiotic-loaded polymers possessing long-term antibacterial activity the kind of polymer–antibiotic interaction play a significant role. In fact, high specificity of interaction together with a large number of antibiotic binding sites on the polymer allows the optimisation of the amount of adsorbed antibiotic. Thus to increase the amount of the adsorbed drug, polyurethanes were synthesized or modified to introduce different functional groups, i.e. acidic and basic groups. Both antimicrobial drugs employed in this study (amoxicillin and rifampin) possess acidic and basic functional groups, which can interact with the polymer matrices. In fact, the amount of antibiotic adsorbed by the here described

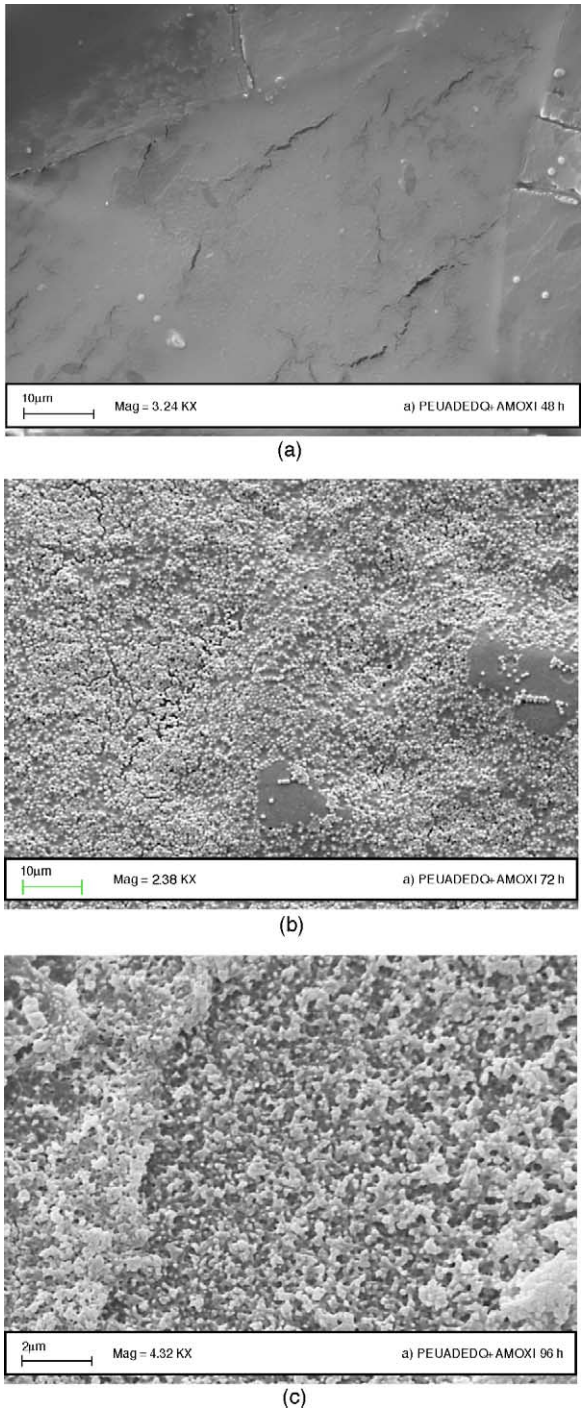


Fig. 5. SEM micrographs showing the *S. epidermidis* (ATCC 12228) growth onto the surfaces of amoxi-treated PEUADEDQ washed for 48 h (a), 72 h (b) and 96 h (c).

Table 5
Inhibition zones of the bacterial growth of the treated-RIF polymers incubated in saline solution or in human serum for increasing times

Time of incubation (days)	Diameter of the inhibition zone (mm)							
	PEUADED		PEUADEDQ		PEUEA		$PEUEASO_3H$	
	Saline solution	Human serum	Saline solution	Human serum	Saline solution	Human serum	Saline solution	Human serum
20	30 ± 1	30 ± 1	30 ± 1	28 ± 1	25 ± 1	26 ± 1	32 ± 1	26 ± 1
60	30 ± 1	20 ± 1	30 ± 1	20 ± 1	18 ± 1	14 ± 1	30 ± 1	18 ± 1

functionalised polyurethanes is particularly elevated with respect to literature data reported for polymers of a different type (Trooskin et al., 1985; Sherertz et al., 1989; Bach et al., 1996). Furthermore, the antibiotic–polymer affinity increases with the introduction in the polymer side-chain of functional groups able to bring about specific interactions. In particular, when the antibiotic–polymer interaction is of the polar type, the amount of bonded antibiotic depends on the matrix hydrophilicity, whereas it is favoured by the presence of strong positive or negative groups on the polymeric surface when an ionic interaction takes place. AMOXI*Na can establish ionic interactions with $PEUEASO_3H$ and with PEUADEDQ, by its primary amino group and its carboxy group, respectively, while AMOXI can only establish "hydrogen bond" interactions with the polymers assayed, even when adsorbed on PEUADED. In fact, the acidic solution (pH = 1.4) used for its adsorption brings about the protonation of the tertiary amino groups of PEUADED that become not available to interact with its carboxy groups. In this latter case the amount of bonded antibiotic depends on the matrix hydrophilicity (Fig. 3a).

As for RIF, it establishes strong acid–base interactions with $PEUEASO_3H$ and PEUADEDQ, due to its tertiary amino group and its phenolic hydroxyl exhibiting acidic properties, weak acid–basic interactions with PEUADED and "hydrogen bond" interactions with PEUEA.

The study of the antibiotic elution with time evidences that only a fraction of the total amount of the initially adsorbed antibiotic is released, and that the eluted amount depends on the strength of the drug–polymer interaction. In fact, the stronger ionic interaction seems responsible for a lower antibiotic release with respect to the hydrogen bond interaction. Moreover, since it was seen that the antibiotic adsorption decreases the swellability of the most hydrophylic polymers (data reported for RIF, Fig. 4b), we concluded that the drug probably penetrates into the underlying layers of the surface, so decreasing the antibiotic release.

The antimicrobial activity of the treated polymers, evaluated in vitro by the Kirby–Bauer test, depends on the amount of the antibiotic adsorbed as well as on the strength of the drug–matrix interaction and on the water swelling of the polymers.

The presence of an inhibition zone of bacterial growth around antibiotic-containing polymeric disks would suggest that the antibiotic was still active and able to diffuse into the culture medium. However, it was observed by optical microscopy and confirmed by SEM observation that the surface of films containing adsorbed antibiotics, differently from the surface of original not treated films, was free from microbial flora, even when, after high washing times, the inhibition zone was no more present. Therefore, we can infer that the drug antimicrobial activity is due not only to the diffusion of its molecules into the aqueous medium (which is the cause of the inhibition zone), but also, in the absence of diffusion, to its presence on the film surface (see Fig. 5). Probably, for longer times the loss antibacterial activity is related to the lack of antibiotic on the polymer surface. Therefore, bacteria can be adhered and form biofilm.

The presence of wide inhibition zones around the RIF treated polymers with respect to that treated with AMOXI might be due to a better ability of RIF to diffuse into the colture medium and to its great antibacterial activity against the *S. epidermidis* ATCC 35984 strain employed. In fact, its MIC is of 0.003 μg/ml versus a value of 35.0 μg/ml for AMOXI.

The antibiotic–polymer system giving the better result was RIF-PEUADEDQ, providing protection from

microbial colonization even after 10 months of washings in saline solution.

Considering that when intravascular catheters are implanted in humans they intimately contact the bloodstream, we evaluated the possible role of serum proteins on the antimicrobial activity exerted by rifampin-treated polymers around the disk. The obtained results evidence that the presence of the serum proteins influences the antimicrobial activity of the polymers. In fact, at the same incubation time, the size of the inhibition zone measured around the disks decreases of about 30%. In spite of this influence, the diameter of the inhibition zone remains large even after few months of serum incubation. We consider this duration time as sufficient for potential clinical applications.

5. Conclusion

In order to reduce intravascular catheter-related infections, some polyurethanes of a new type were coated with antibiotics. The so realized antibiotic–polymer compositions were assayed in their in vitro antimicrobial efficacy.

It was seen that the antibiotic–polymer affinity increases with the introduction in the polymer side-chain of functional groups able to bring about a specific interaction. In particular, when the antibiotic–polymer interaction is of the polar type, the amount of antibiotic bonded depends on the matrix hydrophilicity, while when an ionic interaction takes place, the adsorption is favoured by the presence of strong positive and negative groups on the polymeric surface.

The antibiotic–polymer system giving the best results was RIF-PEUADEDQ, providing protection from microbial colonization even after 10 months of washings in saline solution.

The presence of the serum proteins seems to decrease the antimicrobial activity of the polymers treated with rifampin. In spite of this influence the diameter of the inhibition zone still remains large even after few months of serum incubation

The satisfactory results obtained by RIF adsorption on polymers provided with charged groups make these systems potentially suitable for a number of severe clinical conditions requiring the total protection of patients from medical device-related infections.

The risk of emerging multidrug resistant nosocomial pathogens exists in any situation, including this, where antimicrobials are used. On the other hand, this tool, decreasing the risk of colonization, would avoid an excessive systemic use of antibiotics aimed at preventing device-related infections.

Acknowledgements

This work was supported by the Italian Ministry for University and Research “M.I.U.R.” (National Project).

References

Bach, A., Darby, D., Bottiger, B., Bohrer, H., Motsch, J., Martin, E., 1996. Retention of the antibiotic teicoplanin on a hydromer-coated central venous catheter to prevent bacterial colonization in postoperative surgical patients. Int. Care Med. 22, 1066–1069.

Danese, P.N., 2002. Antibiofilm approaches: prevention of catheter colonization. Chem. Biol. 9, 873–880.

Donelli, G., De Paoli, P., Fadda, G., Marone, P., Nicoletti, G., Varaldo, P.E., 2001. A multicenter study on central venous catheter-associated infections in Italy. J. Chemother. 13, 251–262.

Donelli, G., Francolini, I., 2001. Efficacy of antiadhesive, antibiotic and antiseptic coating in preventing catherter-related infections. J. Chemother. 13, 56–67.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microrganisms. Clin. Microbiol. Rev. 15, 167–193.

Elliott, T., 2000. Intravascular catheter-related sepsis – novel methods of prevention. Int. Care Med. 26, S45–S50.

Heard, S.O., Wagle, M., Vijayakumar, E., McLean, S., Brueggemann, A., Napolitano, L.M., Edwards, L.P., O’Connell, F.M., Puyana, J.C., Doern, G.V., 1998. Influence of triple-lumen central venous catheters coated with chlorhexidine and silver sulfadiazine on the incidence of catheter-related bacteremia. Arch. Int. Med. 158, 81–87.

Kamal, G.D., Divishek, D., Kumar, G.C., Porter, B.R., Tatman, D.J., Adams, J.R., 1998. Reduced intravascular catheter-related infection by routine use of antibiotic-bonded catheters in a surgical intensive care unit. Diagn. Microbiol. Infect. Dis. 30, 145–152.

Mah, T.F., O’Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9, 34–39.

Marciante, K.D., Veenstra, D.L., Lipsky, B.A., Saint, S., 2003. Which antimicrobial impregnated central venous catheter should we use? Modeling the costs and outcomes of antimicrobial catheter use. Am. J. Infect. Control 31, 1–8.

Marconi, W., Francolini, I., Piozzi, A., Di Rosa, R., 2001. Antibiotic releasing urethane polymers for prevention of catheter related infections. Bioact. Compat. Polym. 16, 393–407.

Marconi, W., Galloppa, A., Martinelli, A., Piozzi, A., 1996. New polyurethane compositions able to bond high amounts of both albumin and heparin. II: Copolymers and polymer blends. Biomaterials 17, 1795–1802.

O'Grady, N.P., Alexander, M., Dellinger, E.P., Gerberding, J.L., Heard, S.O., Maki, D.G., Masur, H., McCormick, R.D., Mermel, L.A., Pearson, M.L., Raad, I.I., Randolph, A., Weinstein, R.A., 2002. Guidelines for the prevention of intravascular catheter-related infections. Center for disease control and prevention. MMWR Recomm. Rep. 9, 1–29.

Raad, I.I., Darouiche, R.O., Hachem, R., Abi-Said, D., Safar, H., Darnule, T., Mansouri, M., Morck, D., 1998. Antimicrobial durability and rare ultrastructural colonization of indwelling central catheters coated with minocycline and rifampin. Crit. Care Med. 26, 219–224.

Sheng, W., Ko, W., Wang, J., Chang, S., Hsueh, P., Luh, K., 2000. Evaluation of antiseptic central venous catheters for prevention of catheter-related infection in intensive care unit patients. Diagn. Microbiol. Infect. Dis. 38, 1–5.

Sherertz, R.J., Forman, D.M., Solomon, D.D., 1989. Efficacy of dicloxacillin-coated polyurethane catheters in preventing subcutaneous Staphylococcus aureus infection in mice. Antimicrob. Agents Chemother. 33, 1174–1178.

Trooskin, S.Z., Donetz, A.P., Harvey, R.A., Greco, R.S., 1985. Prevention of catheter sepsis by antibiotic bonding. Surgery 97, 547–551.