With the aim to develop novel antifouling materials, segmented polyurethanes were synthesized with a hard/soft domain structure in which the soft domain is made of different macrodiols. Polyurethanes with polycaprolactide or poly-L-lactide as a soft domain significantly reduced adhesion of *Staphylococcus epidermidis*, probably due to the polymer bulk hydrophobicity and surface wettability. These novel polymers could be useful for the manufacturing or coating of medical devices.

**Keywords**
antifouling coatings; microbial biofilms; polyurethanes; medical devices; infections; *Staphylococcus epidermidis*.

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**Abstract**
In hospital settings, biofilm-based medical device-related infections are considered a threat to patients, the sessile growing bacteria playing a key role in the spreading of healthcare-associated infections. In recent decades, the design of antifouling coatings for medical devices able to prevent microbial adhesiveness has emerged as one of the most promising strategies to face this important issue. In order to obtain suitable antifouling materials, segmented polyurethanes characterized by a hard/soft domain structure, having the same hard domain but a variable soft domain, have been synthesized. The soft domain was constituted by one of the following macrodiols: polypropylene oxide (PPO), polycaprolactide (PCL), and poly-L-lactide (PLA). The effects of the polymer hydrophilicity and the degree of hard/soft domain separation on antifouling properties of the synthesized polyurethanes were investigated. Microbial adherence assays evidenced as the polymers containing PCL or PLA were able to significantly reduce the adhesion of *Staphylococcus epidermidis* with respect to the PPO-containing polymer.

**Introduction**
Medical devices are increasingly used in clinics to administer nutrients and drugs, to monitor the status of patients, or to replace damaged body tissues and organs. However, any medical device implanted in the human body can be colonized by one or more microbial species that, within hours to days, develop as well-organized sessile communities known as biofilms. Thus, according to the increased antibiotic resistance exhibited by biofilm-growing microbial pathogens (Høiby *et al.*, 2010; Mah, 2012) and their high propensity to spread among hospitalized patients, medical device-related infections represent a serious health risk in hospital settings (Al Mohajer & Darouiche, 2012; Donelli & Vuotto, 2014).

The design of antifouling and/or antimicrobial coatings for medical devices is one of the most promising strategies to face this important issue (Francolini & Donelli, 2010; Campoccia *et al.*, 2013). In particular, polymer-based coatings have been the subject of extensive research in recent decades, due to their tunable chemical properties. In fact, a large variety of antimicrobial agent-releasing polymers has been developed by either adsorption or impregnation with metal ions (Francolini *et al.*, 2010; Chernousova & Epple, 2013), antiseptic agents (Rupp *et al.*, 2005; Monzillo *et al.*, 2012), and antibiotics alone (Ramos *et al.*, 2011; Raad...
The poly(1-lactide) diol was synthesized by ring opening polymerization (ROP) by using 1,6-hexanediol as an initiator. Particularly, l-lactide (10 g) was added to a 3-neck glass ampoule and heated at 140 °C under magnetic stirring. Then, 1,6-hexanediol (0.8 g) was added in a 1 : 10 molar ratio with respect to l-lactide in order to obtain a theoretical number-average degree of polymerization (n) of 10. Reaction was carried out at 140 °C for 24 h, under nitrogen flux and in the presence of Sn(Oct)$_2$ (0.3% of the HDO, mol mol$^{-1}$) as a catalyst. Later, the reaction mixture was cooled and chloroform (10 mL) was added to recover the polymer. Finally, after precipitation in water and diethyl ether, poly(l-lactide) diol (PLA) was dried under vacuum for 24 h.

Synthesis of polyurethanes

Carboxylated segmented polyurethanes (PUs) were synthesized by a typical two-step polymerization. The polymer hard phase was constituted by MDI and DHMPA, the latter providing the polymers with carboxylic groups. Instead, the polymer soft phase was constituted by PPO or PCL or PLA as a macrodiol (Fig. 1). To obtain polymers with two carboxylic groups per repeat unit, a 3 : 2 : 1 molar ratio of monomers MDI : DHMPA : macrodiol was employed in the synthesis. The content of soft phase per repeat unit, determined as the soft/(soft+hard) ratio and expressed as molar fraction, was 0.50. To verify the influence of the amount of soft phase in the polymer, we have also synthesized a PU-based polyurethane using a 2 : 1 : 1 monomer molar ratio, so obtaining a polymer with one carboxylic group and a soft phase content of 0.65 per repeat unit.

The synthesis procedure for obtaining the 3 : 2 : 1 PPO-based polyurethane was as follows: MDI (7.5 g) and DHMPA (2.7 g) were dissolved in DMF (15 mL) and allowed to react at room temperature for 30 min under nitrogen flow. After this first condensation step, a macrodiol (11 g) solution in DMF (5 mL) and the catalyst dibutyltin dilaurate (with the
catalyst 1% mol mol$^{-1}$ with respect to macrodiol) were added to the polymerization mixture and kept at 50 °C for 24 h, under nitrogen flow. Then, polymers were recovered by precipitation in basic water (NaOH: polymer-COOH 1 : 4 molar ratio), purified by Soxhlet extraction in ether, and dried under vacuum for 1 day. The obtained polymers, whose structure is reported in Fig. 1, were called as follows: Macrodiol$_n$-UA, where $n$ indicates the content of soft phase per repeat unit, U is for urethane, and A is for acid.

**Thermal analysis**

Differential scanning calorimetry (DSC) analysis was performed from −100 to 200 °C under N$_2$ flux using a Mettler TA-3000 DSC apparatus. The scan rate used for the experiments was 10 °C min$^{-1}$ and the sample weight about 6–7 mg.

**Polymer swelling determination**

To evaluate polymer swelling in water, round-shaped polymer specimens (1 cm diameter and 100 μm thickness) were prepared by solvent casting method. Particularly, a THF polymer solution was layered on Teflon plates and the solvent was evaporated under vacuum. Thus, the kinetics of polymer swelling was studied by dipping the obtained polymer specimens in water at room temperature for increasing times. The percentage of swelling ($G_w$) is given by the following equation:

$$G_w(\%) = \frac{G - G_0}{G_0} \times 100$$

where $G_0$ is the initial weight of the film and $G$ is the weight of the swollen film (dried by a light pressure with filter paper).

**Determination of polymer surface wettability by contact angle measurements**

The contact angle measurements in distilled water were performed using a computerized CAHN DCA 312 dynamic contact angle analyzer. Samples were prepared by casting THF polymer solutions on glass slides. Particularly, previously cleaned glass cover slides were coated with a thin polymer layer by dipping in a dilute polymer solution. Then, in order to obtain a smooth homogeneous surface, the solvent was allowed to evaporate slowly at room temperature and pressure. For the experiments, a stage speed of 70 μm s$^{-1}$ was used. The contact angles in advancing ($\theta_a$) and in receding ($\theta_r$) were obtained as the average of three values measured in the immersion and emersion phases of the sample, respectively.

**Evaluation of polymer antifouling properties by bacterial adhesion experiments**

The ability of the synthesized polyurethanes to control bacterial adhesion was evaluated both by counting the number of CFUs per polymer surface unit and by scanning electron microscopy (SEM). The biofilm-producing *Staphylococcus epidermidis* ATCC 35984 strain, cultured in tryptic soy agar (TSA) and broth (TSB), was employed in the experiments. TSB was supplemented with 1% glucose to promote exopolysaccharide production.

Disks (1 cm diameter and 100 μm thickness) of the synthesized polyurethanes were immersed in 2.5 mL of bacterial suspension (10$^8$ CFUs mL$^{-1}$) in TSB and incubated for 24 h at 37 °C. Then, polymer disks were collected, washed twice with phosphate-buffered saline (PBS, pH = 7.4) to remove loosely adherent cells and either detached to count CFUs per polymer surface unit or fixed for SEM observations.

For detachment of bacteria, polymer disks were put in test tubes with 10 mL of Ringer’s solution, sonicated for 5 min, and vortexed for 10 s to collect bacterial cells. Six 10-fold dilutions were prepared, and three 10 μL aliquots of each dilution were plated on TSA plates and CFUs were counted after 18 h incubation at 37 °C.

For SEM analysis, polymer disks were fixed with 2.5% glutaraldehyde in 0.1 M PBS at room temperature for 30 min. Sample dehydration was performed by dipping samples first in ethanol/water solutions (30/70, 50/50, 70/30, 100)
90/10 and 100/0), each treatment lasting for no more than 2 min for an overall time of 10 min, and then in hexamethyldisilazane for 30 s. Finally, samples were gold-coated by sputtering and observed by scanning electron microscopy.

Statistics
Analysis of variance comparisons were performed using MINTAB. Differences were considered significant for P values of < 0.05. Data were reported as means ± 1 SD.

Results
Four segmented polyurethanes, having the repeat unit composition, soft phase content, and molecular weight reported in Table 1, were successfully synthesized by a two-step polymerization. Particularly, these polymers belong to three different soft phase types: PPO, PCL, and PLA. The first two were commercially available, while the latter being purposely synthesized by us. Two PPO-based polyurethanes with a soft content of 0.50 and 0.65, respectively, were also investigated. The molecular weight of the synthesized PLA monomer, evaluated by 1H-NMR (data not shown), resulted to be 1126 g mol⁻¹.

Thermal analysis
To study the degree of hard/soft phase separation of the four synthesized polymers, the glass transition temperature (Tg), characteristic of the soft phase, was determined (Table 1). The semi-crystalline polymer PCL0.50-UA showed a Tg lower than the three amorphous polymers PPO0.50-UA and PLA0.50-UA and PPO0.65-UA indicating a good hard/soft phase separation, providing large hydrophilic soft domains that are responsible for the greater mobility of the PCL0.50-UA. In fact, it is known that the Tg increases if the soft phase interacts with the hard phase, thus resulting in a low degree of hard/soft phase separation.

Polymer swelling in water
In order to evaluate how the type and content of soft phase influenced polymer hydrophilicity, experiments of water uptake were carried out for each of the four synthesized polyurethanes. From the swelling curves, reported in Fig. 2, the influence of the soft phase type on polymer swelling is largely evident. Particulariy, PLA0.50-UA swelled up to 40% in c. 24 h indicating viscoelastic and hydrophilic properties of this polymer. The low swelling (10%) of PCL0.50-UA is due to the semi-crystalline state of this polymer, different from the other two polymers that are amorphous. In addition, a higher soft phase content of the polymer improved swelling, as shown by the PPO0.65-UA that exhibited a swelling (25%) greater than that of PPO0.50-UA (15%).

Polymer surface wettability
To have an estimation of polymer wettability, contact angle measurements were carried out on polymer-coated glass covers. The advancing (θa) and receding (θr) angles for all the synthesized polyurethanes are reported in Table 1. PPO0.50-UA showed a θa lower than PPO0.65-UA, indicating a greater wettability of the first polymer. The polyurethanes PCL0.50-UA, PLA0.50-UA, and PPO0.50-UA showed similar θa (c. 70°) but different θr. The θa values of PCL0.50-UA and PLA0.50-UA lower than PPO0.50-UA indicated a higher wettability of these two polymers, presumably due to the presence on their surface of large hydrophilic domains able to interact with water.

Bacterial adhesiveness
Antifouling properties of the obtained polymers were studied with regard to their ability to counteract the adhesion of the biofilm-producing S. epidermidis ATCC 35984 strain. The
variation of the type of the soft phase, keeping constant the soft content (0.50), significantly influenced the bacterial adhesiveness, as shown in Fig. 3 where the number of CFUs cm$^{-2}$ is reported. Particularly, the best antifouling properties were displayed by PLA 0.50-UA ($2 \times 10^7$ CFUs cm$^{-2}$) followed by PCL 0.50-UA ($1 \times 10^6$ CFUs cm$^{-2}$) and PPO 0.50-UA ($1 \times 10^5$ CFUs cm$^{-2}$), that is, the higher the surface wettability (lower $h$, Table 1) the lower the bacterial adhesion. On the other hand, bacterial adhesiveness was slightly reduced by the increase in the soft phase content, the polymer PPO 0.65-UA showing only 1-log reduction with respect to PPO 0.50-UA.

These findings were confirmed by SEM analysis (Fig. 4). In fact, after 24-h incubation, a mature S. epidermidis biofilm was observed on PPO 0.50-UA (Fig. 4a) surface. Instead, only single cells or small bacterial clusters were observed on the surface of PCL 0.50-UA at low (500X) and high (3000X) magnification (Fig. 4b and c). Moreover, no bacterial colonization was observed on the surface of PLA 0.50-UA (Fig. 4d).

**Discussion**

Segmented polyurethanes are considered elastomers of choice for the manufacturing of medical devices, particularly intravascular catheters, vascular grafts, and artificial heart-assisting devices. In fact, these polymers possess excellent biocompatibility and high flexibility due to their hard/soft phase separation that can be easily modulated during the synthesis to obtain polymers resistant under stress, that is richer in extended hard domains, or more flexible when the hard domains are less extended than soft domains. The soft phase, being the hydrophilic and flexible region of the polymer, is known to regulate the polyurethane interaction with biologic fluids, cells, and bacteria.

The risk of development of foreign body infections greatly increases soon after the implantation of a medical device due to the deposition on its surfaces of host proteins promoting bacterial adhesion and biofilm formation. As an example, Staphylococcus aureus has been found to possess microbial surface proteins able to specifically bind host proteins such as fibronectin or fibrinogen (Speziale et al., 2009; Geoghegan et al., 2013).

To control protein adsorption and inhibit bacterial adhesion on medical device surfaces, several polysaccharides, including heparin (Abdelkefi et al., 2007), hyaluronic acid (Morra & Cassinelli, 1999), dextran (Perrino et al., 2008), and chitosan (Sagnella & Mai-Ngâm, 2005), have been proposed as hydrophilic coatings.

Other than polysaccharides, hydrophilic synthetic polymers, particularly PEG (Park et al., 1998; Orban et al.,...
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1999; Chen et al., 2009; Rana et al., 2010), were also investigated as antifouling coatings for polyurethane-based medical devices. Although PEG possesses unique properties of nontoxicity and biocompatibility, the main limitation associated with its grafting to the polymer surface is the poor resistance to hydrolysis (Shen et al., 2002), a possible cause of coating detachment.

The development of four new segmented polyurethanes exhibiting intrinsic antifouling properties based on the degree of their hydrophilicity, surface wettability, and hard/soft phase separation was herein investigated. Particularly, we have evaluated how the bulk hydrophilicity and surface wettability were affected by the soft phase type and content and how these properties were able to modulate the S. epidermidis adhesion to the polymers. The four polyurethanes investigated had a common hard domain but a variable soft domain constituted by PLA or PPO or PCL.

Keeping constant the soft/hard ratio (i.e. soft phase content, Table 1), the variation of the soft phase type involved a marked variation of polymer thermal, viscoelastic, and swelling properties. The PLA-based polyurethane was amorphous and able to significantly swell in water (Fig. 2), this last finding indicating viscoelastic properties of this polymer. In fact, there are two kinds of water adsorbed to the polymer: (1) one physically entrapped in the accessible interstices of the polymer and (2) one interacting with polymer functional groups by hydrogen bonds. The first kind of water is mainly responsible for polymer swelling and is high in content in amorphous and viscoelastic polymers.

Particularly, the use of PLA as a soft phase allowed the PLAo.50-UA to reach a 40% value of swelling giving rise to a 5-log reduction in bacterial adhesion per polymer surface unit (CFUs cm⁻²) with respect to PPOo.50-UA (Fig. 3). After 24-h exposure to a 10⁶ CFUs mL⁻¹ bacterial culture, the polymer PL Ao.50-UA did not show any presence of adherent bacteria on its surface (Fig. 4d).

For the PPO-based polyurethanes, we investigated the two with soft content 0.50 (PPOo.50-UA) and 0.65 (PPOo.65-UA). According to the obtained results, a greater soft content in the polyurethane caused a higher polymer swelling in water and a lower number of CFUs per polymer surface unit. In fact, PPO o.65-UA, exhibiting a swelling at the equilibrium of c. 25%, showed a statistically significant 1-log reduction with respect to PPOo.50-UA that exhibited a 15% swelling.

Similar findings were reported by Corneillie et al. (1998), who synthesized segmented polyurethanes with different soft phase type by employing PEG, PPO, and polytetramethylene oxide (PTMO) in different ratios. The reduction in the adhesion of S. aureus and Enterococcus faecalis was found to be most pronounced for the PEO/PPO series that showed higher swelling than the PTMO-based materials because PTMO increased polymer rigidity.

The PCl0.50-UA showed a poor swelling due its semi-crystalline state but a good surface wettability as evidenced by the measured low receding contact angle (Table 1).

In fact, besides polymer swelling ability, also surface wettability is an important feature because the interaction between the polymer and bacteria occurs at the interface. Contact angle measurements allowed us to assess this material property, low values of contact angle indicating hydrophilic features of the polymer surface. In general, the receding contact angle, which is measured in the emersion phase of the sample from water, is influenced more than the advanced contact angle by the strength of interactions between water and hydrophilic domains of the polymer surface. Because the antifouling ability of polymers has been related to the formation on the polymer surface of a tightly bound water layer that hinders the surface adsorption of proteins and bacteria, a low θc can be considered a suitable parameter for predicting the antiadhesive properties of materials.

In fact, PCl-UAo₂ possessing a swelling percentage similar to PPO-UAo₂ (10% vs. 15%) but a greater surface wettability (θc = 41 vs. 50) was much more able to counteract bacterial adhesiveness (2-log reduction in CFUs cm⁻²). PCL-containing polyurethanes were also recently synthesized by Ma et al. (2013) to be employed as antifouling coatings for underwater marine applications. In this study, to decrease polymer crystallinity, polyglycolide was also introduced in the soft phase together with PCL. Results showed an increase in polymer wettability (lower value of advancing and receding contact angles) with a consequent improvement of polymer antifouling ability.

Summarizing, the PLA- and PCL-containing polyurethanes possessed good antifouling properties that seem to be related to both polymer bulk hydrophilicity and surface wettability. Particularly, the newly synthesized PL Ao.50-UA, possessing the highest swelling percentage and the lowest θc, was able to completely inhibit S. epidermidis adhesion after 24-h exposure.

The application of this polyurethane either for the manufacturing or the coating of medical devices could contribute to a significant reduction in the incidence of related infections, the most innovative feature of this polymer being represented by its intrinsic ability of counteracting biofilm formation without any specific surface functionalization or impregnation with antimicrobial agents to release.

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References


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