

The synthesis of polymeric dual-functional antimicrobial surface based on poly(2-methyl-2-oxazoline)

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Abstract

There is a high interest in the development of antimicrobial coatings to fight bacterial infections. We present the development of dual-functional antimicrobial surface, in which a biopassive platform was functionalized with bioactive compounds on the surface, using a graft copolymer system poly(L-lysine)-graft-poly(2-methyl-2-oxazoline)-quaternary ammonium compound (PLL-g-PMOXA-QAC). Alkyne functionality was introduced to the PMOXA chain at α -terminus by initiating the living cationic polymerization of 2-methyl-2-oxazoline with a propargylic-initiator. The reaction was terminated with carboxy derivative-terminator that allows grafting of the polymeric chain from the β -terminus to poly(L-lysine) (PLL) backbone, resulting in graft copolymer alkynyl PLL-g-PMOXA. The conjugation between alkynyl PLL-g-PMOXA and QAC was then performed using click reaction. The chemical structures of the polymers were characterized by MALDI-TOF spectrometry and NMR spectroscopy. The results demonstrate that we have successfully synthesized PLL-g-PMOXA-QAC copolymer with grafting density (number of lysine/number of PMOXA) of 0.33. The resulting PLL-g-PMOXA-QAC copolymer was then immobilized onto carboxylated tissue cultured polystyrene (TCPS) surface and exposed to bacteria solution to test its dual-functional properties. Preliminary live-and-dead bacteria study indicates dual-functionality of the PLL-g-PMOXA-QAC-coated surface.

Keywords: biopassive, bioactive, PLL-g-PMOXA-QAC, dual-functional, *click* reaction

Introduction

The development of a biofilm on a medical device and the inherent difficulties of its suppression have wide-ranging consequences for the patients. Among them, severe pain, need for explantation, and requirement for a revision surgery are the most common ones (Schneider *et al.*, 2008). Intra-vascular catheter-related infections are also a major problem and cause of morbidity and mortality. The annual infection rate for cardiovascular implants is even higher (7.4%). Bacterial contamination is also a widespread problem in the population

using devices such as contact lenses, dental implant materials, intrauterine contraceptive devices and vaginal tampons. It is also a problem related to the safety of tools and materials used in medicine or surgery. There is therefore a great interest in approaches to prevent or reduce the risk of infections related to implanted materials.

Currently, several approaches have been developed to protect biomaterials from bacterial contamination (Xie *et al.*, 2011). Based on the type of interaction with bacteria, one may distinguish between 'biopassive' and 'bioactive' biomaterial surfaces. Biopassive surfaces prevent the adhesion of bacteria but do not actively interfere with them. In contrast, bioactive surfaces aim at killing bacteria upon contact or at interfering with the quorum sensing between bacteria preventing further proliferation and maturation of biofilms. A

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further distinction can be made between stably surface-immobilized antimicrobials and active release systems. Appropriate choice of systems depends on the type of antimicrobials and their mechanism of action. Regulatory aspects are also to be considered, in particular in regard to whether approval for a medical device of drug is required.

Materials having biopassive (non-fouling) properties prevent the adhesion of microorganisms and the formation of biofilms. Various hydrophilic polymers are used as protein-resistant coatings. Among them, poly(ethylene glycol) (PEG) has been the most frequently investigated polymer to impart biopassivity to surfaces (Konradi *et al.*, 2012). Nevertheless, several limitations of PEG-based technology have been reported. One of them is loss of biopassive function in case of long-term application. It is believed that PEG degrades by auto-oxidation due to the repetitive oxygen atom in its structure (Pidhatika *et al.*, 2012, Chen *et al.*, 2014).

An alternative polymer with a peptide-like structure is poly(2-oxazoline) (Hoogenboom, 2009). In particular, we were interested in poly(2-methyl-2-oxazoline) (PMOXA). Studies on poly(oxazoline) (POx) grafted to liposomes and micelles (Lee *et al.*, 2003) did not report any adverse effects of the polymer in animal models thus suggesting that POx behave similar to PEG for this type of conjugates. Moreover, this polymer, with brush conformation on surfaces, was recently reported as an excellent non-fouling polymeric coating against serum-protein adsorption and bacteria adhesion (Pidhatika *et al.*, 2010). Series of brush-like copolymers consisting of a polycationic poly(L-lysine) (PLL) backbone and PMOXA side chains (PLL-g-PMOXA) have been immobilized on Nb₂O₅-surface and proven to have very similar protein-repellent properties when compared to the extensively studied (PEG)-based system (PLL-g-PEG) (Pasche *et al.*, 2003). In terms of stability, PMOXA that mimics polypeptide structure was found to be significantly more stable compared

to PEG with polyether structure (Pidhatika *et al.*, 2012). However, biopassive coating may deteriorate during longer-term contact with bacteria or body fluid in general due to mechanical defects of the layer and secretion of oxidative substances from bacteria, requiring other strategies to explore.

There have been attempts to prevent device-related infections by immobilizing bioactive antimicrobial compounds on surfaces. Quaternary ammonium compounds (QAC) are the most frequently studied antimicrobial compounds immobilized on various surfaces. Bouloussa *et al.* (2008) immobilized QAC on hydroxylated surfaces like glass, silicon wafers and cellulose and demonstrated that QAC could kill 99.7%-100% of Gram-negative and Gram-positive bacteria. Brizzolaro and Stamper (2007) covalently immobilized QAC on flat glass substrates and 1- μ m-diameter silica microparticles respectively, and investigated the antibacterial activity of these surfaces against *Staphylococcus aureus* (*S. aureus*). Klibanov and co-workers (Klibanov, 2007, Nebojscarona *et al.*, 2005, Tiller *et al.*, 2001) showed high activity of QASs on flat surfaces. The antibacterial activity of QAC is induced by the mechanical contact between the ammonium cation and the anionic bacterial membrane, resulting in disruption of the membrane system (Gottenbos *et al.*, 2002).

Here we present the development of novel dual-functional (biopassive and bioactive) biocompatible surface coatings as model systems for testing their functionality towards the prevention of biomaterial-related infections. Polyelectrolyte co-polymer brush composed of poly(2-methyl-2-oxazoline) chains grafted to poly(L-lysine) backbone (PLL-g-PMOXA) serves as a biopassive protective layer on the surface. When hydrated, it prevents the non-specific adsorption of biologically active species. Further, introduction of bioactive quaternary ammonium compound (QAC) into the biopassive copolymer brush provides dual functionality of the coating with the aim of

increasing its antimicrobial efficiency and reducing the extent of dead bacteria fouling. The coupling of the QAC to the PLL-g-PMOXA copolymer matrix is performed by copper(I)-catalyzed cycloaddition reaction (*click* reaction). This reaction proceeds in a variety of solvents, tolerates a wide range of pH values, and performs well over a broad temperature range. It is quantitative, stereospecific and highly tolerant towards different functional groups. *Click* reaction is defined as the Cu(I)-catalyzed cycloaddition between terminal acetylenes and azides, resulting in 1,4-disubstitution of 1,2,3-triazole (Bock *et al.*, 2006).

Materials And methods

Materials

Poly(L-lysine) (PLL) was purchased from Sigma-Aldrich. Initiator prop-2-ynyl-4-methylbenzenesulfonate (propargyl tosylate) was purchased from Fluka and used as received. Monomer 2-methyl-2-oxazoline (MOXA) was purchased from Acros Organics and distilled under reduced pressure right before used with KOH was used as a drying agent. Terminating reagent ethyl piperidine-4-carboxylate (4-piperidine ethyl ester, PipEtEst) was purchased from Acros Organics and purified by vacuum distillation. The obtained distillate was stored under argon at -4°C. HPLC grade Acetonitrile (ACN) was purchased from Fluka Chemicals and freshly dried by refluxing over CaH₂ under a dry argon atmosphere. Azido quaternary ammonium compound N₃-C₃H₆-N⁺-C₁₂H₂₅ was provided from custom synthesis. Catalysts for carbodiimide chemistry, N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were purchased from Pierce and Sigma Aldrich, respectively. Dialysis tubing was purchased from Spectrum Laboratories, Inc.

Ultrapure water was used for dialysis, rinsing step, and making buffer. Ultrapure water was produced in a Milli-Q system

Gradient A 10 from Millipore (Zug, Switzerland). The system is equipped with Elix 3, which removes 95 - 99% of inorganic ions, 99% of dissolved organic compounds, bacteria, and particulates.

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and other chemicals used for buffer preparation were purchased from Fluka (Buchs, Switzerland). During the entire study, HEPES solution with additional 150 mM NaCl was used. The buffer solution was adjusted to pH = 7.4 using 6 M NaOH.

Synthesis of PLL-g-PMOXA-QAC

The synthesis route of PLL-g-PMOXA-QAC is shown in figure 1. Carboxy-terminated PMOXA was first prepared by living cationic ring opening polymerization (Konradi *et al.*, 2008, Von Erlach *et al.*, 2011). The 2-methyl-2-oxazoline (8.5 ml, 100 mmol; freshly distilled from KOH through a vigreux column) was dissolved in acetonitrile (30 ml; freshly distilled from CaH₂) under argon. Prop-2-ynyl-4-methylbenzenesulfonate (2 mmol; used as received) was added at 0°C under argon. The mixture was then heated to 70°C for 20 h. Subsequently, the reaction mixture was cooled to room temperature and the reaction was quenched by addition of ethyl piperidine-4-carboxylate (6 mmol; distilled through a vigreux column) for 12 h. The solvent was removed under reduced pressure to yield clear colorless oil. After addition of water (MilliQ, 18.3 MΩ cm), the polymers were purified by dialysis in a 1000 MWCO tubing for 2 × 24 h. C₃H₃-PMOXA-COOC₂H₅ was collected as a white solid (4 g, ~50%) after lyophilization.

In the second step, the carboxy ethyl ester (-COOC₂H₅) end group was hydrolyzed by reaction with sodium hydroxide solution (NaOH_(aq)) at pH = 14 and room temperature for 24 h, resulting in C₃H₃-PMOXA-COOH. The polymers were purified and lyophilized using the same procedure as described above.

The C₃H₃-PMOXA-COOH polymers were then grafted onto PLL backbone

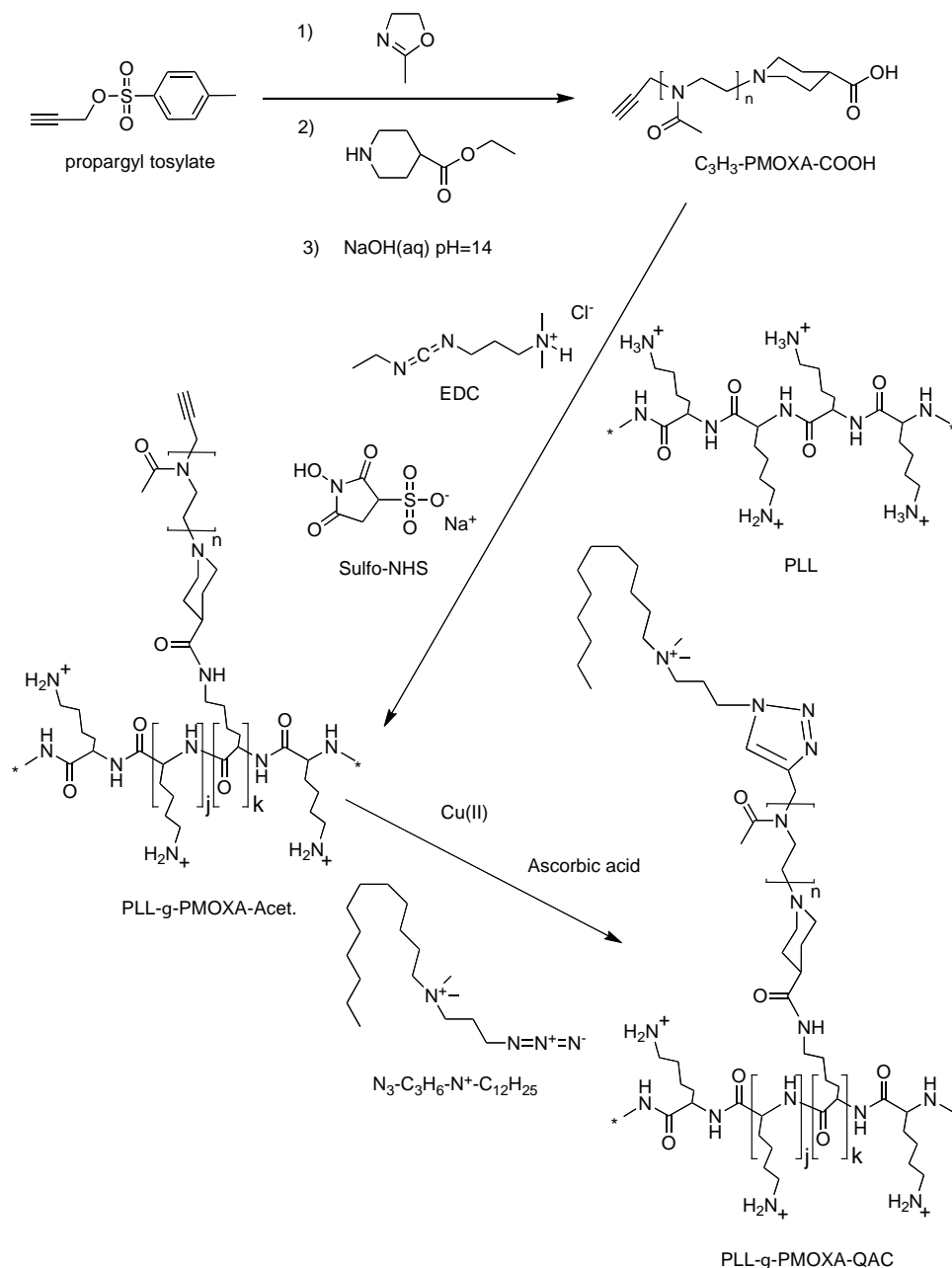


Figure 1. Synthesis route for the preparation of alkyne PLL-g-PMOXA-QAC.

(poly(L-lysine) hydrobromide, MW 15000 - 30000 g/mol) in aqueous buffer solution of pH = 7.4 (10 mmol/l HEPES) using water soluble carbodiimide chemistry. PLL, C_3H_3 -PMOXA-COOH, Sulfo-NHS And EDC were individually dissolved in HEPES buffer. PLL solution was then mixed with C_3H_3 -PMOXA-COOH solution, followed by Sulfo-NHS, And finally EDC solution. Sulfo-NHS was

equimolar And EDC was 10-fold excess with respect to the carboxylic acid function. Final concentrations were 3 - 30 mg/ml PLL-HBr, 35 - 70 mg/ml C_3H_3 -PMOXA-COOH, 8 - 16 mmol/l Sulfo-NHS And 80 - 160 mmol/l EDC. The mixtures were stirred for 15 h at room temperature and then transferred to dialysis tubes having a 12000 - 14000 MWCO. Dialysis was performed for 2 × 24 h

against water followed by lyophilization. The molecular weight cut off was chosen such that both, low molecular weight reagents And unreacted C_3H_3 -PMOXA-COOH were effectively removed. Alkynyl PLL-g-PMOXA was obtained as white powder with typical yields on the order of 80% (200 mg).

In the final step, the alkynyl PLL-g-PMOXA copolymer (20 mg, 1 eq) was reacted with azido quaternary ammonium compound $N_3-C_3H_6-N^+-C_{12}H_{25}$ (0.374 mg, 10 eq) using *click* chemistry method in a 1:1 H_2O/t -BuOH solvent, with Cu(II) And Na-ascorbate as the catalysts. The *click* reaction was performed at room temperature for 24 hours, followed by dialysis, lyophilisation, And characterization.

The synthesized polymers were characterized using matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF) spectrometry And nuclear magnetic resonance (NMR) spectroscopy. MALDI-ToF measurements were performed on a Bruker Daltonics Ultraflex II instrument, while NMR spectroscopy was performed on a 500 MHz Bruker Ultrashield Avance instrument.

Surface modification

The PLL-g-PMOXA-QAC copolymers were then adsorbed onto carboxylated tissue cultured polystyrene (TCPS) surfaces (24 well plates). These surfaces expose a net negative charge, while the lysine units from the PLL backbone that are not bound to PMOXA expose a net positive charge in physiological pH. This condition results in electrostatic attachment between the TCPS surfaces And the PLL-g-PMOXA-QAC copolymers. Polymer films were prepared by dip and rinse procedure. Briefly, the polymers were dissolved at 0.1 mg/ml concentration in a filtered (0.22 μ m) HEPES buffer solution containing 10 mM HEPES supplemented with 150 mM NaCl And adjusted to pH 7.4 (HEPES2). Then, 500 μ l of copolymer solutions were placed onto the freshly opened carboxylated tissue cultured polystyrene (TCPS) surfaces, completely

covering their surfaces. Polymer adsorption was allowed to proceed for minimum 2 h, followed by extensive washing with HEPES2 solution, ultrapure water, and finally by blow-drying under a stream of nitrogen.

Bacteria culture

Bacteria *E. coli* K12 AAEC191A that express green fluorescence protein (eGFP) were grown overnight in LB media, supplemented by antibiotics, at 37°C And 100 rpm of shaking. The eGFP expression was then induced by adding IPTG with 1 mM final concentration, followed by incubation at 30°C And 100 rpm of shaking, for minimal 3-4 h. The bacteria were washed with M9 minimal media supplemented with antibiotics And IPTG, and the bacteria concentration was adjusted to 10^9 bacteria/ml.

Bacteria assay And live-And-dead staining

The PLL-g-PMOXA-QAC-coated TCPS surfaces were exposed to freshly prepared bacteria solution (10^9 bacteria/ml) at room temperature. The volume of bacteria solution was 500 μ l per TCPS well. Following 1 h of exposure, the surfaces were washed thoroughly with M9 minimal media supplemented with antibiotics And IPTG, keeping the surfaces immersed in the solution at all times. Propidium iodide stain was then added to the solution after washing with 30 μ M final concentration. Live and dead surface-adherent bacteria were imaged using an inverted Nikon TE2000-E microscope equipped with a Hamamatsu Photonics EM CCD-C9100 camera using Metamorph software (Molecular Devices, Inc.).

Results And discussions

MALDI-ToF spectrometry was performed to determine the molecular weight of PMOXA. Figure 2 shows the MALDI-ToF spectrum of (C_3H_3) -PMOXA- $(COOC_2H_5)$ ($M_n = 4400$ g/mol, $DP = 50$, $PDI = 1.01$). All detected peaks presented a peak-to-peak average distance equal to 85 g/mol, which confirmed the repeating

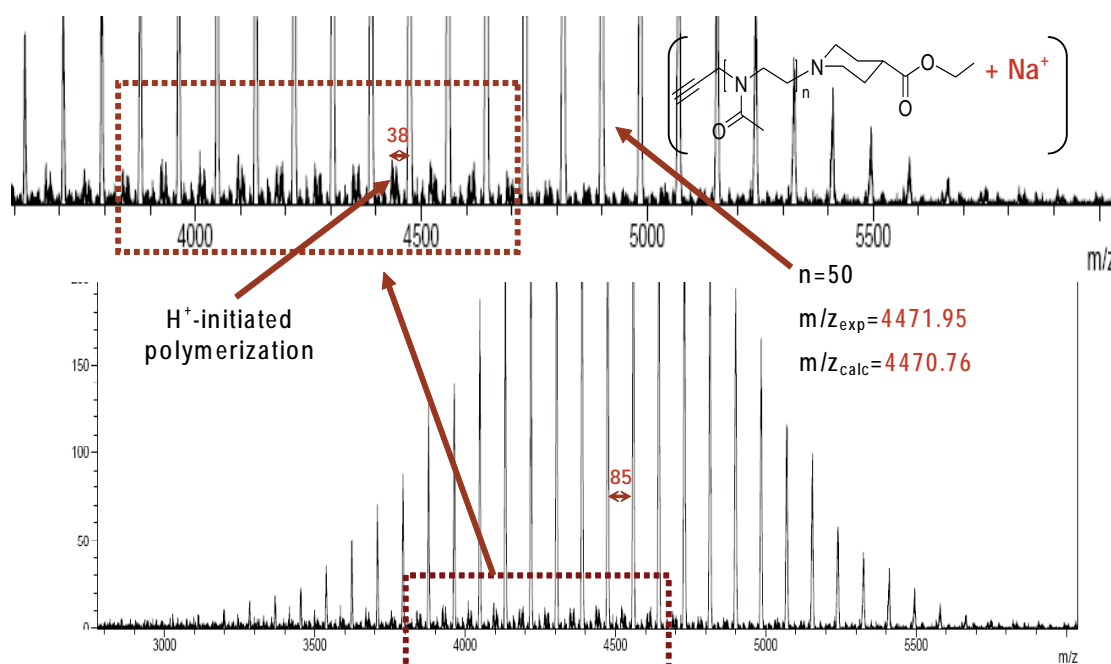


Figure 2. MALDI-ToF spectrum of (C_3H_3) -PMOXA- $(COOC_2H_5)$ ($M_n = 5000$ g/mol, $DP = 50$, $PDI = 1.01$).

unit of 2-methyl-2-oxazoline (MOXA). The main peaks show the molecular weight of the corresponding chemical species coupled with a sodium ion.

In addition to the main peaks, second minor peaks with lower masses relative to the main peaks at 38-mass differences were detected. For MALDI-ToF spectra of poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline), this phenomenon has been observed earlier and could be attributed to an initiation of a small fraction of monomers with a proton (H^+) instead of the initiator (Gaertner *et al.*, 2007, Jordan *et al.*, 2001). Thus, a minor part of the polymer presents as (H) -PMOXA- $(COOC_2H_5)$ instead of (C_3H_3) -PMOXA- $(COOC_2H_5)$. However, the intensity of this minor distribution is low and the molar mass of the resulting polymer is not significantly affected.

Proton (1H) NMR spectroscopy was performed to confirm the chemical structure of the synthesized polymers and graft copolymers. Figures 3a shows the NMR spectra of (C_3H_3) -PMOXA- $(COOC_2H_5)$, before and after hydrolysis of the ester group.

The peaks located between 3.3 - 3.8 ppm ($-CH_2-N(CH_3-C=O)-$, peak D) and between 1.8 - 2.3 ppm ($-(C=O)-CH_3$, peak E) are typical for a PMOXA chain, as previously reported by (Gaertner *et al.*, 2007). Furthermore, peaks located between 4.1 - 4.3 ($-(C=O)O-CH_2-CH_3$ that overlaps with $-(CH_3-C=O)N-CH_2-C-CH_3$, peak A,B) and between 1.2 - 1.4 ($-CH_2-CH_3$, peak C) could be assigned to the ester group at the β -end of PMOXA chains.

The modification of functionality at β -end group after hydrolysis, i.e. transformation of (C_3H_3) -PMOXA- $(COOC_2H_5)$ into (C_3H_3) -PMOXA- $(COOH)$, could be clearly identified by the modification of peak at 4.0 - 4.2 ppm (peak A), and disappearance of the peak at 1.2 - 1.4 ppm (peak C) (Figure 3b). The degree of polymerization (DP) was then calculated as $(I_{-(CH_2-N(CH_3-C=O)-, \text{ peak D})} / 2) / I_{-(CH_3-C=O)N-CH_2-C-CH_3, \text{ peak A}}$ which results in a DP value of 48, considerably close to the DP value obtained from MALDI-ToF experiment.

The (C_3H_3) -PMOXA- $(COOH)$ was then coupled to a PLL backbone presenting $-NH_2$ along its chain, by using carbodiimide chemistry in aqueous solution. The 1H

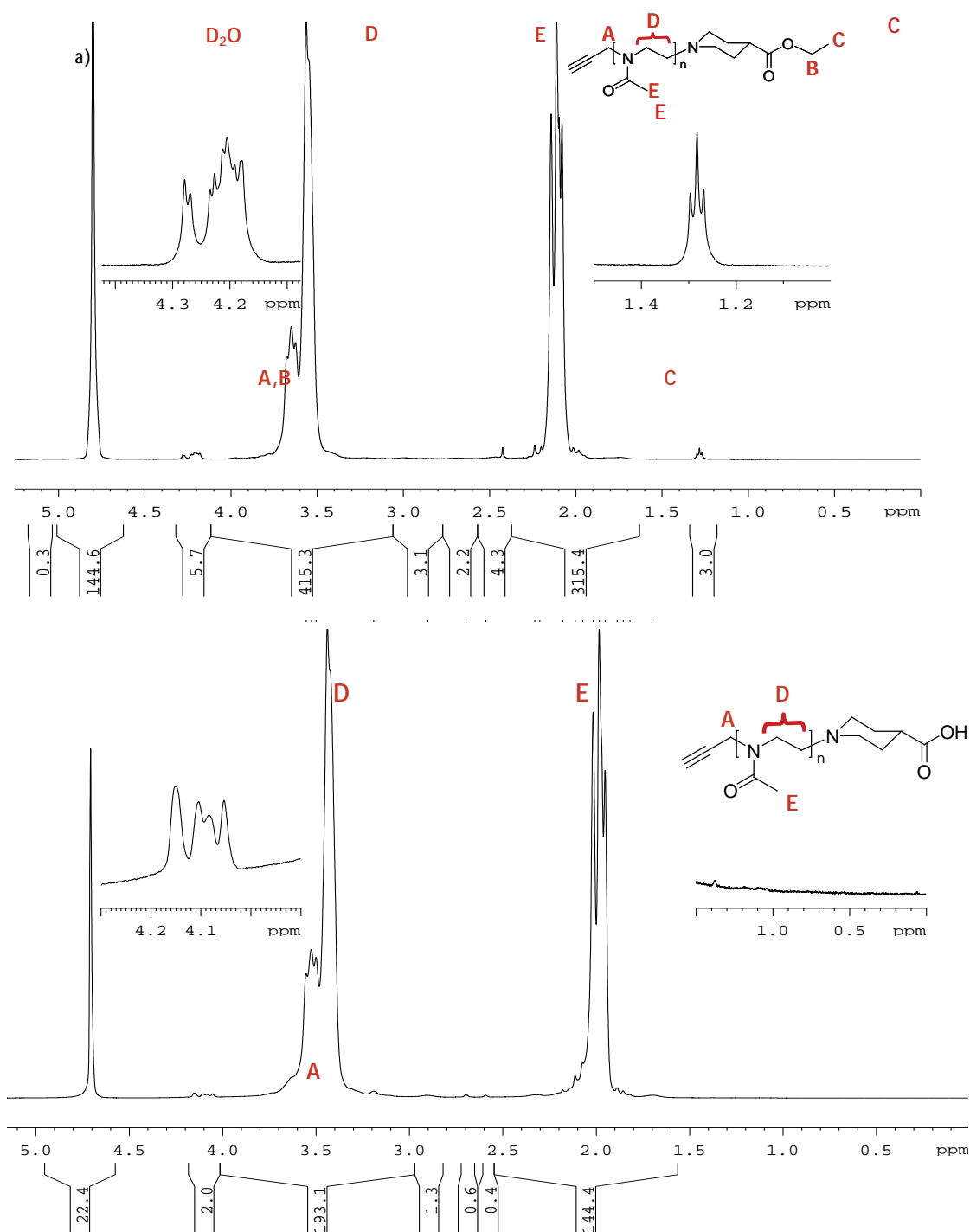


Figure 3. ¹H NMR spectrum of (C₃H₃)-PMOXA-(COOC₂H₅) in D₂O (at 500MHz) a) before And b) after hydrolysis of the ester group.

NMR spectra of alkynyl PLL-g-PMOXA is presented in figure 4. Upon grafting (amide formation), changes of peak intensities were observed in the chemical shifts at 4.2, 3.05, 2.9,

and between 1.2 - 1.8 ppm. Grafting density α was calculated from the ratio between peak intensity of -CH₂- groups adjacent to amide And amine, respectively. Thus, $\alpha = I_{-CH_2-N(C=O)-}$

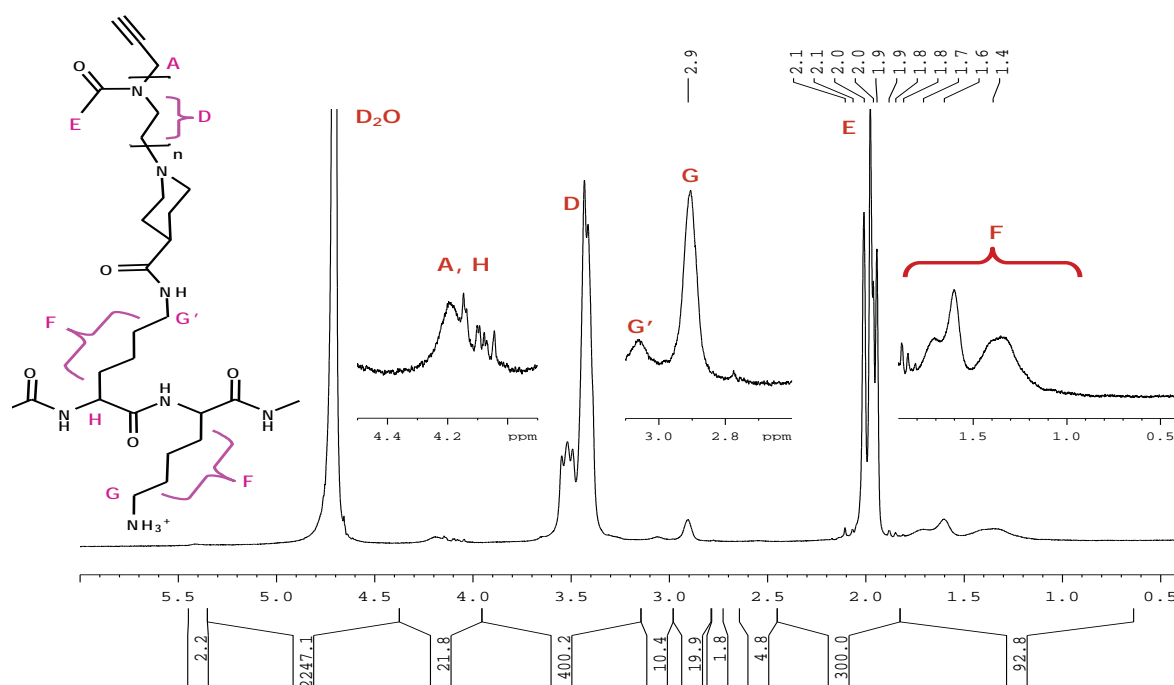


Figure 4. ¹H NMR spectra of alkynyl PLL-g-PMOXA ($\alpha=0.33$) in D₂O (at 500MHz).

$/ (I_{-CH_2-N(C=O)-} + I_{-CH_2-NH_3^+}) = I_{G'}/ (I_{G'} + I_G)$, which results in α value of 0.33. We have previously found that this value is the optimum grafting density value for PLL-g-PMOXA brush as biopassive surface coating (Pidhatika *et al.*, 2010, Pidhatika *et al.*, 2012).

Figure 5 shows the NMR spectrum of PLL-g-PMOXA-QAC and the assignment of peaks. The successful conjugation between PLL-g-PMOXA and QAC could be seen by the appearance of QAC peaks in addition to the PLL-g-PMOXA peaks, although some overlap were observed.

The ideal conformation of the copolymers on the surface is illustrated in figure 6. The anchor (PLL backbone) serves positive charges from the lysine groups that are not connected to the PMOXA side chains while the surface (TCPS) serves negative charges. The PLL-g-PMOXA-QAC copolymer is thus immobilized on the TCPS surface by electrostatic interactions. The bioactive part (QAC) serves as antimicrobial compound that kills (lyses) approaching bacteria cells to the surface. The biopassive part (PMOXA) serves as anti-adhesive layer that repels

microorganisms And biomolecules including the residues from dead bacteria And the lysate.

The dual-functional antimicrobial surface was then exposed to bacteria solution in order to test its antimicrobial properties. PLL-coated And bare TCPS surfaces served as controls. Figure 7 shows the microscopy images after live-And-dead staining assays on the surfaces. Green cells indicate the presence of living bacteria, whereas red cells indicate dead bacteria on the surface.

It is observed in figure 7 that PLL-coated surface was fully covered with living bacteria. At physiological pH, PLL-coated surface presents positive charges originating from the positively charged free lysine units, causing electrostatic interactions with negatively charged bacteria cells (Pidhatika *et al.*, 2010). In contrary to PLL-coated surface, bare carboxylated TCPS surface presents negative charges, thus a decrease in number of adherent bacteria was observed due to repulsive interaction between similarly (negative) surface charges.

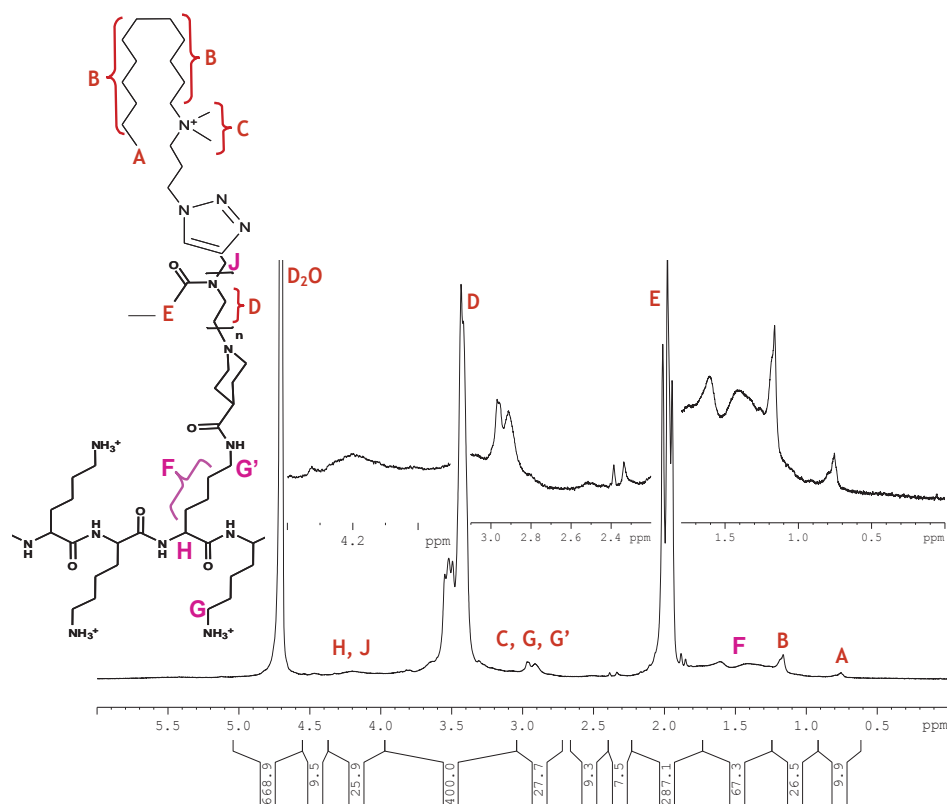


Figure 5. ¹H NMR spectra of PLL-g-PMOXA-QAC in D₂O (at 500MHz).

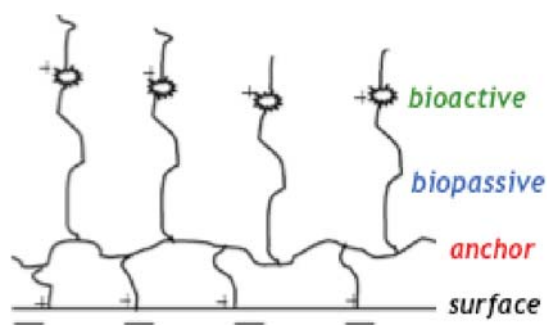


Figure 6. Ideal conformation of PLL-g-PMOXA-QAC copolymer on TCPS, which results in dual-functional antimicrobial surface.

However, several living bacteria managed to adhere on the bare surface due to the absence of steric stabilization and entropic penalty to inhibit strong, irreversible interactions. Interestingly, PLL-g-PMOXA-QAC-coated surface shows very few dead bacteria. This phenomenon indicates the dual-functionality of the surface, i.e. the bioactive compound

presumably kills (interferes) the bacteria approaching the surface, and the biopassive layer repels both living bacteria and protein residue from dead bacteria.

Conclusions

We have successfully synthesized graft copolymer PLL-g-PMOXA ($\alpha = 0.33$) with acetylene functionality as a biopassive platform in the development of dual-functional antimicrobial surfaces. The acetylene functional groups served as the binding sites for the conjugation between the PLL-g-PMOXA and bioactive QAC using *click* reaction. The NMR spectrum of the resulting PLL-g-PMOXA-QAC indicated successful conjugation between PLL-g-PMOXA and QAC. The successful conjugation was further confirmed by the results of preliminary dual-functionality test by means of live-and-dead bacteria assay.

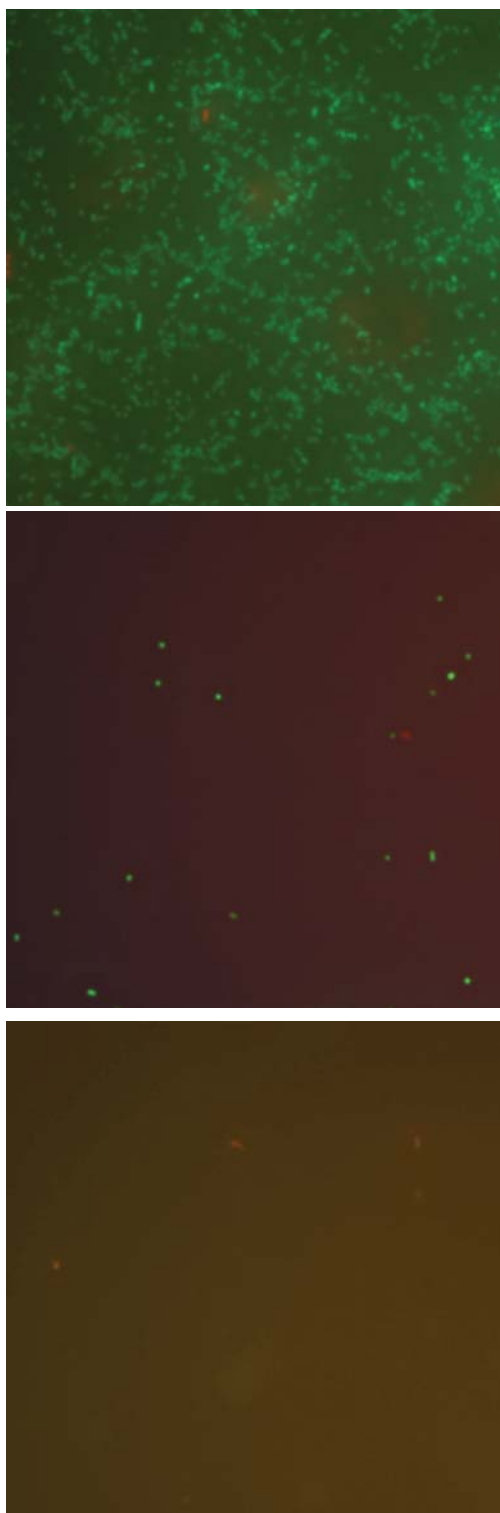


Figure 7. Microscopy images of PLL-coated (above), PLL-g-PMOXA-QAC-coated (middle), and bare TCPS (below) surfaces after exposure of the surfaces to bacteria solution followed by dead-and-live staining assays. The size of each image is 200 x 200 μm .

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References

- Bock, V. D., Hiemstra, H. And Van Maarseven, J. H., 2006. CuI-catalyzed alkyne-azide "click" cycloadditions from a mechanistic and synthetic perspective. *European J of Organic Chem*, 2006, 51-68.
- Bouloussa, O., Rondelez, F. And Semetey, V., 2008. A new, simple approach to confer permanent antimicrobial properties to hydroxylated surfaces by surface functionalization. *Chem Comm*, 951-953.
- Brizzolara, R. A. And Stamper, D. M., 2007. The effect of covalent surface immobilization on the bactericidal efficacy of a quaternary ammonium compound. *Surface and Interface Analysis*, **39**, 559-566.
- Chen, Y., Pidhatika, B., Erlach, T. V., Konradi, R., Textor, M., Hall, H. And Lühmann, T., 2014. Comparative assessment of the stability of nonfouling poly(2-methyl-2-oxazoline) and poly(ethylene glycol) surface films: an in vitro cell culture study. *Biointerphases*, **9**, 031003-1-031003-10.
- Gaertner, F. C., Luxenhofer, R., Blechert, B., Jordan, R. And Essler, M., 2007. Synthesis, biodistribution And excretion of radiolabeled poly(2-alkyl-2-oxazoline)s. *Journal of Controlled Release*, **119**, 291-300.
- Gottenbos, B., Van Der Mei, H. C., Klatter, F., Nieuwenhuis, P. And Busscher, H. J., 2002. In vitro And in vivo antimicrobial activity of covalently coupled quaternary

- ammonium silane coatings on silicone rubber. *Biomaterials*, **23**, 1417-1423.
- Hoogenboom, R., 2009. Poly(2-oxazoline)s: a polymer class with numerous potential applications. *Angewandte Chemie, International Edition*, **48**, 7978-7994.
- Jordan, R., Martin, K., Rader, H. J. and Unger, K. K., 2001. Lipopolymers for surface functionalizations. 1. Synthesis and characterization of terminal functionalized poly(N-propionylethylenimine)s. *Macromolecules*, **34**, 8858-8865.
- Klibanov, A. M. 2007. Permanently microbicidal materials coatings. *Journal of Materials Chemistry*, **17**, 2479-2482.
- Konradi, R., Acikgoz, C. And Textor, M., 2012. Polyoxazolines for nonfouling surface coatings - a direct comparison to the gold standard PEG. *Macromolecular Rapid Communications*, **33**, 1663-1676.
- Konradi, R., Pidhatika, B., Muehlebach, A. and Textor, M., 2008. Poly-2-methyl-2-oxazoline: a peptide-like polymer for protein-repellent surfaces. *Langmuir*, **24**, 613-616.
- Lee, S. C., Kim, C., Kwon, I. C., Chung, H. And Jeong, S. Y., 2003. Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(ϵ -caprolactone) copolymer as a carrier for paclitaxel. *Journal of Controlled Release*, **89**, 437-446.
- Nebojscarona, M. M., Wang, J., Lewis, K. and Klibanov, A. M., 2005. Immobilized N-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed. *Biotechnology and Bioengineering*, **90**, 715-722.
- Pasche, S., Depaul, S. M., Voros, J., Spencer, N. D. And Textor, M., 2003. Poly(L-lysine)-graft-poly(ethylene glycol) assembled monolayers on niobium oxide surfaces: a quantitative study of the influence of polymer interfacial architecture on resistance to protein adsorption by ToF-SIMS And *in-situ* OWLS. *Langmuir*, **19**, 9216-9225.
- Pidhatika, B., Möller, J., Benetti, E. M., Konradi, R., Rakhmatullina, E., Muehlebach, A., Zimmermann, R., Werner, C., Vogel, V. And Textor, M., 2010. The role of the interplay between polymer architecture And bacterial surface properties on the microbial adhesion to polyoxazoline-based ultrathin films. *Biomaterials*, **31**, 9462-9472.
- Pidhatika, B., Rodenstein, M., Chen, Y., Rakhmatullina, E., Muehlebach, A., Acikgoz, C., Textor, M. And Konradi, R., 2012. Comparative Stability Studies of Poly(2-methyl-2-oxazoline) And Poly(ethylene glycol) Brush Coatings. *Biointerphases*, **7**, 1-15.
- Schneider, O. D., Loher, S., Brunner, T. J., Schmidlin, P. And Stark, W. J., 2008. Flexible, silver containing nanocomposites for the repair of bone defects: antimicrobial effect against *E. coli* infection And comparison to tetracycline containing scaffolds. *J. Mater. Chem.*, **18**, 2679-2684.
- Tiller, J. C., Liao, C. J., Lewis, K. And Klibanov, A. M., 2001. Designing surfaces that kill bacteria on contact. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 5981.
- Von Erlach, T., Zwicker, S., Pidhatika, B., Konradi, R., Textor, M., Hall, H. And LüHmann, T., 2011. Formation And characterization of DNA-polymer-condensates based on poly(2-methyl-2-oxazoline) grafted poly(L-lysine) for non-viral delivery of therapeutic DNA. *Biomaterials*, **32**, 5291-5303.
- Xie, X., Möller, J., Konradi, R., Kisielowe, M., Franco-Obregón, A., Nyfeler, E., Muehlebach, A., Chabria, M., Textor, M., Lu, Z. And Reimhult, E., 2011. Automated time-resolved analysis of bacteria-substrate interactions using functionalized microparticles And flow cytometry. *Biomaterials*, **32**, 4347-4357.