Int. J. Bio-Inorg. Hybr. Nanomater., 11(3): 119-126, Autumn 2022

Synthesis and characterization of Poly(2-oxazoline)–Cefixime conjugates based surface-attached thin film of non-fouling hydrogels

Sh. Mohammadi Ziari[1] and G. Bagheri[2*]

[1] Department of Pharmaceutical Chemistry, Shahryar Branch, Islamic Azad University, Shahryar, Iran

[2] Department of Chemical Engineering, Shahryar Branch, Islamic Azad University, Shahryar, Iran

Received: 9 February 2022; Accepted: 12 April 2022

ABSTRACT: CDDS controlled drug release systems are a new tool to release drugs in the human body and have found many applications in the field of pharmaceutical science and technology. Therefore, the main goal of the present research is to use the surface thin layer attached to polyoxazoline from non-precipitating hydrogels for the release of Cefixime antibiotic. In this study, poly(2-ethyl-2-oxazoline) (PEOXA) chains containing 11 mole percent of benzophenone molecules were synthesized to form a hydrogel polymer network on the substrate. Important variables for the production of hydrogel film with high gel content and stability, such as heat treatment to remove the solvent, UV wavelength (which determines the radiation energy) and energy input dose and their effect on the gel content were investigated. The AFM morphology image showed a homogeneous polymer film with an average roughness of 30 nm. Cefixime is often taken every 12 hours, of which 6 hours are spent releasing the drug, and the next 6 hours are related to its effectiveness. Examining the in vitro release of Cefixime on the hydrogel substrate showed that the synthesized hydrogel has suitable properties for Cefixime drug release.

Keywords: *Cefixime, Drug release, Hydrogel, Surface fouling, Poly(2-oxazoline), Thin film.*

INTRODUCTION

Surface fouling on biomedical devices is ubiquitous, cost demanding, and can be life-threatening [1]. Coating of biomedical devices with non-fouling film has so far been one of the most attractive strategies in the prevention of surface fouling [1]. Some pre-requisites for polymers that can be used for the fabrication of non-fouling surface coatings are hydrophilicity (the polymers should be hydrophilic), charge (the polymers should carry net neutral charge), and Hydrogen-bond groups (the polymers should contain H-bond donor but should not contain H-acceptor groups) [2–5]. Among a broad spectrum of polymers that fulfill the mentioned prerequisites are poly(ethylene glycol) [6–8], phosphorylcholine [9, 10], polysaccharide [11–13], poly(vinylpyrrolidone) [14], poly(vinyl alcohol) [15], polyacrylamides [5, 16] and polyacrylates [17], and the poly(2-oxazoline)s [18, 19]. For the latest, there has been a significant emergence of this class of polymer since the first reports on poly(2-oxazoline)s (POXs) around 5 (five) decades ago due to their excellent bio-

(*) Corresponding Author - e-mail: bagheri.gita@yahoo.com

compatibility and stealth behavior, in which they do not readily trigger foreign body response [20]. The reason for this may come from the similarity between POXs and peptides in terms of chemical structure, in which they consist of Carbon-Carbon-Nitrogen at their main chains. Poly(2-methyl-2-oxazoline) (PMOXA) for example, it offers isomerism to poly (homo alanine) peptide chain. The peptide-like structure offers not only biocompatibility, but also resistance to auto oxidation and thus high stability [21, 22]. Surface grafting of POXs have been performed on different inorganic and organic substrates using various methods to modulate their interfacial, physicochemical properties and provide functions to the modified surface. In particular, the performance of POX coatings as bio interfaces to hinder nonspecific biological adhesion has been extensively explored [23]. Grafted poly(2-methyl-2-oxazoline) (PMOXA) significantly reduced non-specific adsorption of serum proteins on Nb_2O_5 surfaces [24] and increased stability of DNA particles against serum proteins and enzymatic DNase-I digestion [21]. Good transfection efficiency combined with low cytotoxicity of the PMOXA-grafted DNA particles was demonstrated in COS-7 cells [21]. In comparison to PEG brush film, the golden standard in the area, PMOXA brush film was significantly more stable upon long term exposure to biological environment [22, 25]. Morgese and Benetti [23] reviewed a plethora of grafting strategies of PAOXAs on to surfaces, and concluded that poly(2-alkyl-2-oxazoline) s (PAOXAs) have been one of the most promising materials among the possible solutions for biocompatible, non-fouling surface coatings [23]. Among the grafting strategies are the reaction between catechol PAOXAs and metal oxides [26], xanthane-PAOXAs and citrate-Au [27]. The resulting network, however, carries high positive charge, not satisfying the prerequisites for non-fouling surface coatings. In the present work, a non-fouling film of poly(2-oxazoline)-based polymer network (hydrogel) on surfaces is developed by partially functionalizing poly(2-ethyl-2-oxazoline) (PEOXA) chains with benzophenone units that react with any C-H bonds in the vicinity upon UV illumination and allow for a direct and simple immediate attachment to the substrate, regardless whether it is a (hydrophobically-modified) metal or metal oxide, or a polymer. The polymer network thus covalently bounds to the surfaces of the substrates and have a much higher thickness and robustness compared to monolayer.

MATERIAL AND METHODS

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated. The biotinylated-laminin and Cy5- streptavidin were purchased from Cytoskeleton, Inc. and VWR, respectively.

Synthesis of poly(2-ethyl-2-oxazoline)

The synthesis was performed following a previously published protocol [27]. Poly(2-ethyl-2-oxazoline) (PEOXA) with Mw~50,000 g/mol, DP ~ 500, and PDI ~3–4 was purchased from Sigma-Aldrich (CAS 25805–17-8, catalog no. 372846 ALDRICH). PEOXA (5 g) and HCl (16.8%, 105 ml) were added to a 250 ml round-bottom flask. The mixture was stirred and refluxed at 100 °C. After 40 min., the mixture was cooled down to room temperature. Then, neutralization using KOH solution was performed until neutral pH (~7) is reached. The neutral solution was transferred to a dialysis membrane with MWCO ~ 14,000 g/mol (Carl Roth, catalog no. 1784.1) and dialyzed against aquadest bath for at least 2 × 24 h, with changing of the aquadest bath at least every 24 h.

Synthesis of poly(2-ethyl-2- oxazoline)

The synthesis was performed following a previously published protocol [28]. PEOXAm% EI (4 g, 4.7 mmol EI monomer) and tert-amyl alcohol (20 ml) were added to a 50-ml round-bottom flask and stirred until the polymer powder is dissolved. Then, K_2CO_3 (0.85 g) and 4- (bromomethyl) benzophenone 96% (1.36 g) were added to the mixture. The mixture was stirred and heated up to 95 °C. After an overnight reaction, the mixture was cooled down to room temperature, and the solvent, tert-amyl alcohol, was exported using a rotary vaporizer. The (yellowish, oily) solid residue was re-dissolved in dichloromethane. The PEOXA-m%EIBP polymer was then precipitated in excess amount of n-hexane. After vacuum-filtration, the polymer was collected and vacuum-dried for

several days to remove traces of dichloromethane and n-hexane. Film thickness, chemical composition and lifetime of benzophenone molecules were determined. The copolymer was spin-coated (600 rpm, 100 s) from a 50 mg/ml solution in ethanol onto a 1.5 × 1.5 cm Silicon (Si) wafer, previously modified using 3-thoxybenzophenonesilane, for both film thickness and XPS measurements. The film thickness was then measured using ellipsometry and atomic force microscopy (AFM) methods, while the chemical composition on the Si wafer surface was measured using XPS. The same copolymer solution was spincoated on a quartz substrate for benzophenone lifetime measurement. From time to time between 0 to 132 min, the sample on quartz substrate sample was taken out from the Stratalinker and the change in absorbance intensity was monitored using UV/Visible spectroscopy.

Drug loading

0.2 grams of Cefixime drug is dissolved in 50 ml of one liter of 0.3 M hydrochloric acid solution and we put the hydrogels in the solution at a constant temperature for 24 hours. 10 mg of hydrogel is placed in 10 ml of distilled water and then the solution is filtered using a 0.2 micrometer syringe filter and the absorbance of the solution is measured by a spectrophotometer at a maximum wavelength of 286 nm. Using the standard absorption curve and the obtained equations, the concentration of the solution and drug was calculated. Considering that the amount of the primary drug in the solution is determined by the difference between these two values, the amount of the drug enclosed in the hydrogel was obtained. Then using the obtained information and using the following formula, the percentage of loaded drug was calculated [29].

In vitrodrug release

In order to measure the amount of drug release, the dialysis bag method was used. For this purpose, 10 mg of medicinal polymer hydrogel in different concentrations along with 2 ml of phosphate buffer were poured into a 7 kDa dialysis bag that was soaked in deionized water the night before and then immersed in 100 ml of phosphate buffer. And it was placed in a shaking incubator at a temperature of 37 degrees Celsius. At certain time intervals, 1 ml of the environment around the bag was removed and replaced with 1 ml of fresh phosphate buffer. The samples were filtered with 0.2 µm and their absorbance was measured at 286 nm wavelength and using the standard absorbance curve, drug concentration and as a result the amount of free drug in phosphate buffer solution was obtained. The measurement of the released drug was continued until there was no change in the concentration of the solution. For accuracy, all experiments were repeated three times under identical conditions.

RESULTS AND DISCUSSION

The presence of residual secondary amine group, protonated-, and quaternized amine groups, if any, cannot be detected from the NMR spectra. Following the synthesis and NMR characterization, the copolymer is

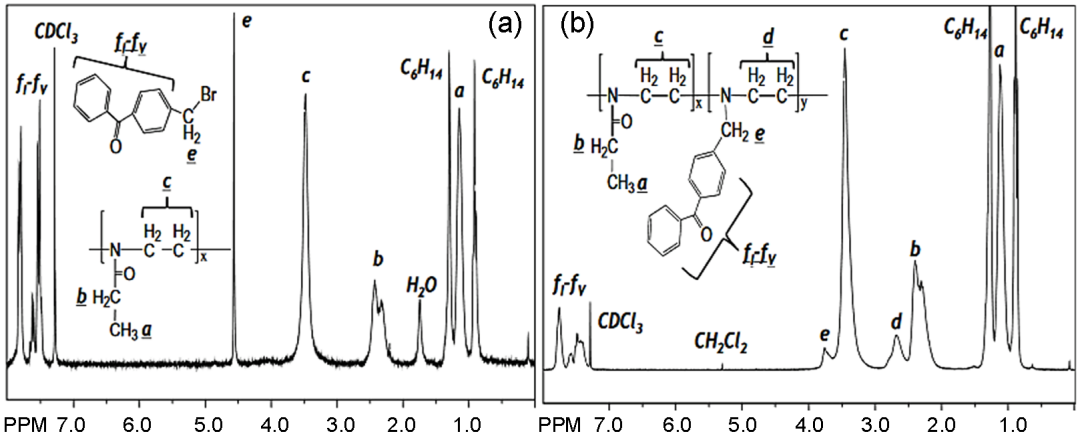

Fig. 1. NMR spectra of PEOXA

immobilized on the substrate surfaces and PEOXA-based hydrogel is generated. One of important parameters in controlling the process and rating the quality of hydrogels (polymer networks) is the gel content. To this, an approximately 1000 nm-thick of copolymer is spin-coated onto Si wafers that are pre-functionalized with 3 ethoxybenzophenonesilane. Several process parameters during the preparation of the network are then varied and the gel content is determined. In purification step, the copolymer is dissolved in dichloromethane and precipitated in n-hexane. The NMR characterization (Fig. 1) shows that residual dichloromethane (CH_2Cl_2) and n-hexane (C_6H_{14}) solvents cannot be totally removed from the copolymer powder. In addition, coating the copolymer onto the substrates employs ethanol as the PEOXA-11%EIBP's solvent. Such organic solvent molecules can also react through C, H-insertion reaction, but this will not connect two copolymer chains and thus not result in increase of the crosslink density, an important factor that influences the gel content.

XPS experiments are then performed to obtain chemical information of the modified surfaces. The XPS samples are prepared following the same procedure as described in the gel content measurements, using an energy dose of 4 J/cm^2 at 365 nm. Fig. 2a, b, c, and d show the survey spectra of bare Si wafer, spin-coated PEOXA-11%EIBP film before any further treatments, spin-coated PEOXA 11%EIBP film after CHiC process and extraction, and spin-coated PEOXA 11%EIBP film after CHiC process, extraction, and incubation in PBS, respectively. Compared to the spectra of bare Si wafer in Fig. 2a, the domination of C 1s and N 1s signals, as well as disappearance of Si 2s and Si 2p peaks on the spectra of PEOXA-11%EIBP-coated wafer in Fig. 2c and d demonstrates the formation of a stable surface-attached copolymer film that provides a sufficient shielding for the Si surface. In agreement with the above-described explanation on gel content and impurities, Fig. 2b shows the presence of (minor) bromide-containing species, originated from the synthesis step, on the PEOXA-11%EIBP-coated surface before any extraction and incubation of the substrate. The bromide signals, however, disappear after extraction and incubation steps (Fig. 2c and d). It is worthwhile to note that the incubation in

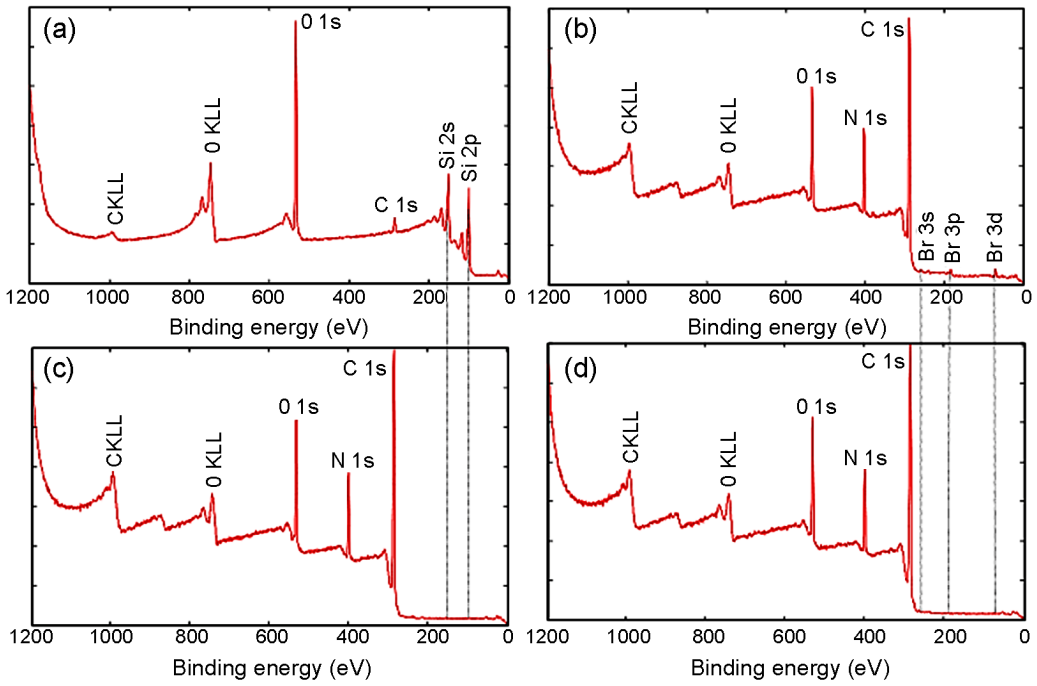

Fig. 2. The survey XPS spectra of (a) bare Si wafer, (b) PEOXA-11%EIBP on Si wafer after spin-coating, (c) PEOXA-11%EIBP on Si wafer after UV light irradiation and extraction using PEOXA-11%EIBP-dissolving solvent, and d PEOXA-11%EIBP on Si wafer after UV light illumination, extraction, and 14-days incubation in PBS buffer. The C, N, and O atomic concentrations obtained from spectra (d) calculated from the integrated area of C1s, N1 s, and O1s peaks are 75, 13, and 12%, respectively.

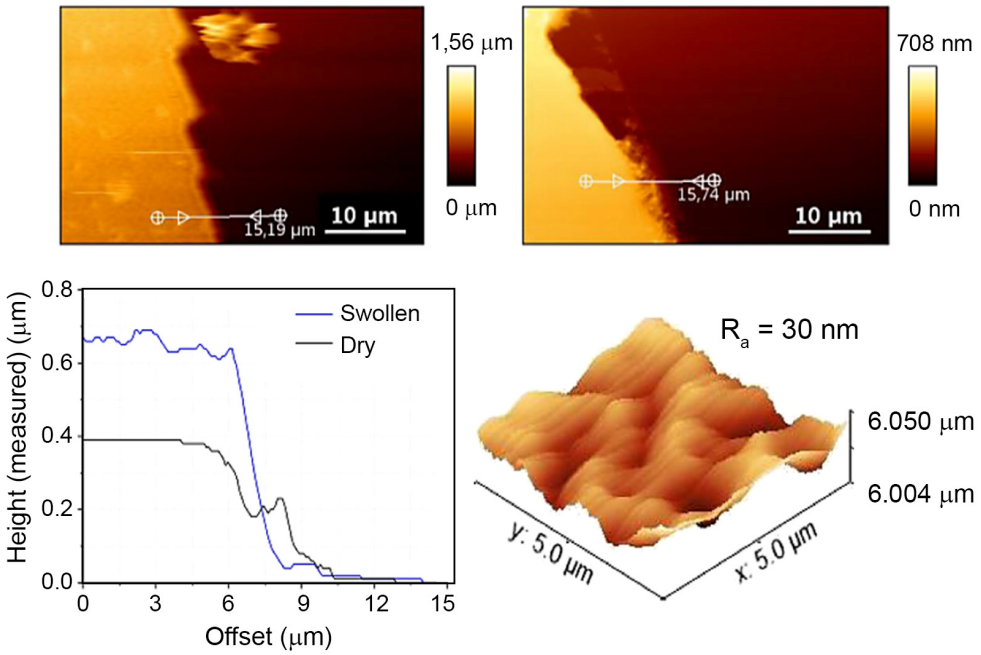

Fig. 3. The AFM data of PEOXA-11%EIBP film after extraction of non-crosslinked materials from the surface followed by incubation of the polymer network in PBS buffer for 14 days.

PBS buffer for 14 days is performed to test the film stability, since exposure to physiological environment during application may lead to copolymer degradation and/or detachment of the copolymer film from the surface. Elemental analysis on the surface enables the determination if the copolymer undergoes any degradation reactions that change its chemical composition. Based on the chemical structure of PEOXA-11%EIBP shown in Fig. 1, the theoretical C, N, and O atomic concentrations exclusively originate from the copolymer are 76, 12, and 12%, respectively, while the corresponding values based on the XPS spectra in Fig. 2d are 75, 13, and 12%. The similar (almost identical) theoretical and experimental atomic concentration values indicate that copolymer degradation is unlikely in this context. In literature, it has been reported that surface attached poly(2-methy-2-oxazoline) in brush configuration is chemically stable upon exposure to HEPES buffer [30]. The XPS data in the present work are thus in agreement with the literature and validate the chemical stability of poly(2-oxazoline) upon exposure to physiological buffer.

An important prerequisite for a non-fouling film in the context of biomedical devices is the hydrophilicity of the film. In this study, the dry and swollen thickness of PEOXA-11%EIBP film on Si wafer is measured using AFM after 14 days of incubation in PBS buffer. A swelling factor is then determined to demonstrate the film hydrophilicity. The topographic images, measured height profiles, and surface morphology from AFM measurements are presented in Fig. 3a, b, c, and d. It is seen that the swollen and dry thickness values are approximately 660 and 390 nm, respectively. According to eq. 3, these values result in a swelling factor of 1.7, which is in an excellent agreement with the reported swelling factor of surface-attached poly(dimethylacrylamide) with similar benzophenone molar concentration along the polymer chain (10%) [27]. The influence of crosslinker content to the swell-

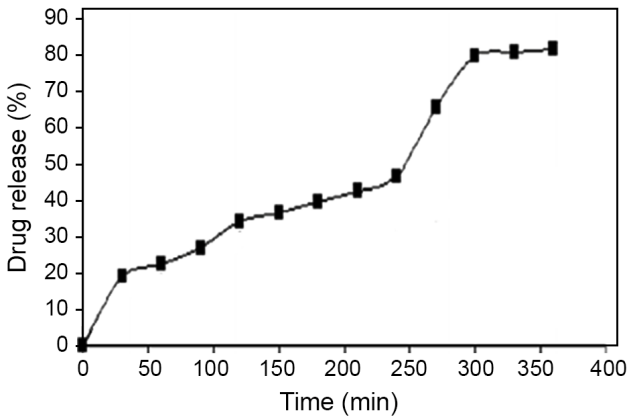

Fig. 4. Drug release(%)

ing behavior of surface-attached hydrogel has been discussed in the literature [28]. In the present study the hydrophobic nature of benzophenone molecules should render the PEOXA chains less hydrophilic and thus less swelling capability. In addition, a high crosslinking density leads to a more compact polymer network and restricted movement of polymer chains, preventing the penetration of water molecules into the network [29]. In Fig. 3d, the surface morphology image shows the presence of a satisfactorily homogenous PEOXA-11%EIBP film on the Si wafer, with an average roughness 30 nm.

Fig. 4. It shows the drug release curve in terms of time. As it is clear in the figure, the release of the drug is done in a time interval of 350 per minute, which is about 6 hours. But Cefixime is often taken every 12 hours, 6 hours of which are spent releasing the drug and the next 6 hours are related to its effectiveness. The results of this part of the research with the results of the research at pH=7.2, the Hi carboxyl group is free and this arrangement leads to an increase in the efficiency without space inside the polymer hydrogel which also causes.

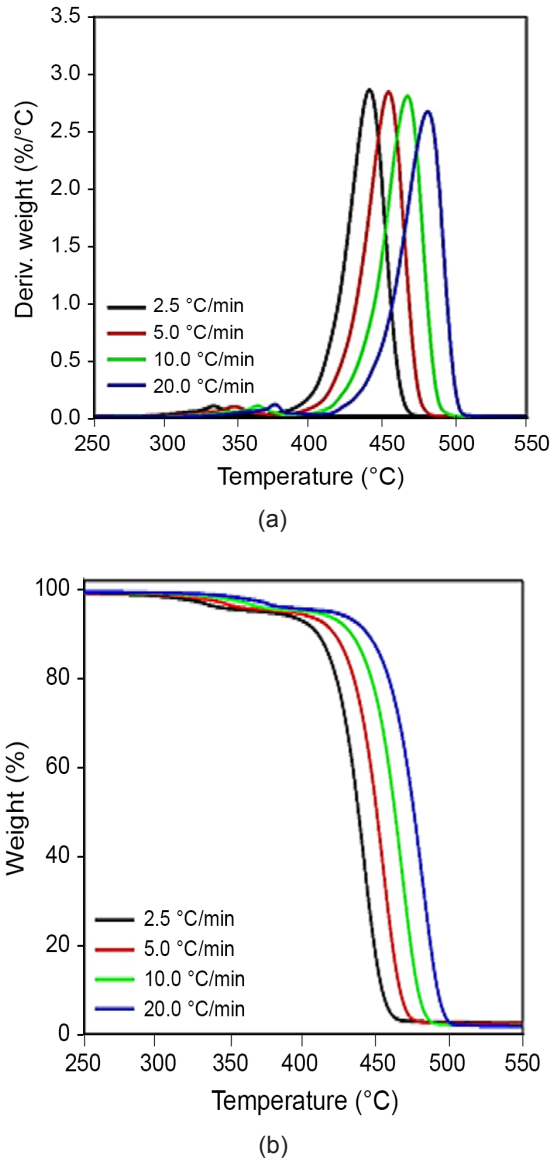

Fig. 5. Thermal degradation analysis of the sample with Cefiximeloading under different heating conditions (a) TGAand (b) DTG.

Fig 5. shows the thermal properties of hydrogel degradation with Cefixime loading. As the picture shows, the sample has two peaks, the first peak at a temperature of 300-400°C (corresponding to the temperature rate) and the second peak, which is a longer peak, at a temperature higher than 450-550°C (corresponding to the rate temperature) created, so from this figure, it can be concluded that the hydrogel destroyed at the beginning is destroyed in the next step of the drug. Reviewing Hoffendam's paper in 2010 investigated polylysine hydrogel loaded with antibiotics. In this research, the reason for this can be seen as the reduction of particle size. Because with the increase of surface energy, the grains become finer and finer grains increase the interface between the grains.

CONCLUSION

Important variables for the production of hydrogel film with high gel content and stability, such as heat treatment to remove the solvent, UV wavelength (which determines the radiation energy) and energy input dose and their effect on the gel content were investigated. The stability of the hydrogel film was studied. The thickness, lifetime of the hydrogel, and the chemical composition of the film were determined using ellipsometry, UV/Vis spectroscopy, and XPS methods, respectively. On a film exposed to physiological buffer for 14 days, XPS results showed that no chemical degradation of the copolymer took place. However, the results showed that the part of the detached film and the remaining thickness depended on the energy input dose during hydrogel preparation. It was shown that a PEOXA-based hydrogel bound to the surface is stable when appropriate conditions are

applied during preparation. Approximately 78% of the gel content and 75-90% stability can be generated on the surface after 30 days of incubation in physiological buffer. The dry and swollen thickness of the stable surface-adhered film measured from AFM experiments showed a swelling factor of 1.7. In addition, the AFM morphology image showed a homogeneous polymer film with an average roughness of 30 nm. Cefixime is often taken every 12 hours, of which 6 hours are spent releasing the drug, and the next 6 hours are related to its effectiveness. The results of the electron microscope show that the grain size of the current research is 212±2. By investigating the in vitro release of Cefixime on the hydrogel substrate, it was shown that the synthesized hydrogel has suitable properties for the release of the Cefixime drug.

REFERENCES

[1] Nguyen, K.T., West, J.L. (2002). Photopolymerizable hydrogels for tissue engineering applications. Biomaterials, 23, 4307–4314.

[2] Chapman, R.G., Ostuni, E., Liang, M.N., Meluleni, G., Kim, E., Yan, L., Pier, G., Warren, H.S., Whitesides, G.M. (2001). Polymeric thin films that resist the adsorption of proteins and the adhesion of Bacteria. Langmuir, 17(4), 1225–1233.

[3] Hoffmann, J., Groll, J., Heuts, J., Rong, H., Klee, D., Ziemer, G., Moeller, M., Wendel, H.P. (2006). Blood cell and plasma protein repellent properties of Star-PEG-modified surfaces. J. Biomater. Sci. Polym. Ed., 17(9), 985–996.

[4] Otsuka, H., Nagasaki, Y., Kataoka, K. (2004). Characterization of aldehyde-PEG tethered surfaces: influence of PEG chain length on the specific biorecognition. Langmuir, 20(26), 11285–11287.

[5] Mahdavi, H., Norouzian, S. (2018). Preparation and characterization of modified ultrafiltration nylon 6 membrane modified by poly(acrylamide-co-maleic anhydride). J. Polymer Res., 25(10), 222.

[6] Krsko, P., Libera, M. (2005). Biointeractive hydrogels. Materials Today (Oxford United Kingdom), 8(12), 36–44.

[7] Koh, W.G., Revzin, A., Simonian, A., Reeves, T., Pishko, M. (2003). BioMEMs materials and fabrication technology: control of mammalian cell and bacteria adhesion on substrates micropatterned with poly(ethylene glycol) hydrogels. Biomed Microdevices, 5(1), 11–19.

[8] Park, J.H., Bae, Y.H. (2003). Hydrogels based on poly(ethylene oxide) and poly(tetramethylene oxide) or poly(dimethyl siloxane). II. Physical properties and bacterial adhesion. J. Appl. Polymer Sci., 89(6), 1505–1514.

[9] Lewis, A.L. (2000). Phosphorylcholine-based polymers and their use in the prevention of biofouling. Colloids Surf. B Biointerfaces, 18(3,4), 261–275

[10] West, S.L. (2003). The biocompatibility of crosslinkable copolymer coatings containing sulfobetaines and phosphobetaines. Biomaterials, 25(7–8), 1195–1204.

[11] Li, P., Wang, J., Lu, W.C., Sun, H., Huang, N. (2005). Surface characterization and antibacterial evaluation of poly(ethylene terephthalate) modified by chitosan-immobilization. Key Eng. Mater., 288- 289, (Advanced Biomaterials VI), 331–334.

[12] An, Y.H., Farino, M., Kang, Q.K., Demcheva, M.V., Vournakis, J. (2005). Glucosamine coating for inhibiting bacterial adhesion to titanium surfaces. Key Eng. Mater., 288-289. (Advanced Biomaterials VI), 343–346.

[13] Morra, M., Cassineli, C. (1999). Non-fouling properties of polysaccharide-coated surfaces. J. Biomater. Sci. Polymer Ed, 10(10), 1107–1124.

[14] Tunney, M.M., Gorman, S.P. (2002). Evaluation of a poly(vinyl pyrrolidone) coated biomaterial for urological use. Biomaterials, 23, 4601–4608.

[15] Koziarz, J., Yamazaki, H. (1999). Stabilization of polyvinyl alcohol coating of polyester cloth for reduction of bacterial adhesion. Biotechnol. Tech., 13(4), 221–225.

[16] Chen, H., Chen, Q., Hu, R., Wang, H., Newby, B.M.Z., Chang, Y., Zheng, J. (2015). Mechanically strong hybrid double network hydrogels with antifouling properties. J. Mater. Chem. B, 3(27), 5426–5435.

[17] Chen, H., Zhao, C., Zhang, M., Chen, Q., Ma,

J., Zheng, J. (2016). Molecular understanding and structural-based Design of Polyacrylamides and Polyacrylates as antifouling materials. Langmuir, 32(14), 3315–3330.

[18] Hoogenboom, R. (2007). Poly(2-oxazoline) s: alive and kicking. Macromol. Chem. Phys., 208(1), 18–25.

[19] Hoogenboom, R. (2009). Poly(2-oxazoline)s: a polymer class with numerous potential applications. Angew Chem. Int. Ed, 48(43), 7978–7994.

[20] Hoogenboom, R. (2017). 50 years of poly(2-oxazoline)s. Eur. Polym. J., 88, 448–450.

[21] von Erlach T., Zwicker, S., Pidhatika, B., Konradi, R., Textor, M., Hall, H., 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 herapeutic DNA. Biomaterials, 32(22), 5291–5303.

[22] Pidhatika, B., Rodenstein, M., Chen, Y., Rakhmatullina, E., Mühlebach, A., Acikgöz, C., Textor, M., Konradi, R. (2012). Comparative stability studies of poly(2-methyl-2-oxazoline) and poly (ethylene glycol) brush coatings. Biointerphases, 7(1), 1–15.

[23] Morgese, G., Benetti, E.M. (2017). Polyoxazoline biointerfaces by surface grafting. Eur. Polymer J., 88, 470–485.

[24] Pidhatika, B., Möller, J., Benetti, E.M., Konradi, R., Rakhmatullina, E., Mühlebach, A., Zimmermann, R., Werner, C., Vogel, V., 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(36), 9462–9472.

[25] Chen, Y., Pidhatika, B., von Erlach, T., Konradi, R., Textor, M., Hall, H., 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(3), 031003.

[26] Morgese, G., Causin, V., Maggini, M., Corrà, S., Gross, S., Benetti, E.M. (2015). Ultrastable suspensions of Polyoxazoline-functionalized ZnO single nanocrystals. Chem. Mater., 27(8), 2957–2964.

[27] De la Rosa, V.R. (2015). Colorimetric logic gates based on poly(2-alkyl-2-oxazoline)-coated gold nanoparticles. Adv. Funct. Mater, 25(17), 2511–2519.

[28] Mansfield, E.D.H., de la Rosa, V.R., Kowalczyk, R.M., Grillo, I., Hoogenboom, R., Sillence, K., Hole, P., Williams, A.C., Khutoryanskiy, V.V. (2016). Side chain variations radically alter the diffusion of poly(2-alkyl-2-oxazoline) functionalized nanoparticles through a mucosal barrier. Biomater. Sci., 4(9), 1318–1327.

[29] Koshkina, O., Lang, T., Thiermann, R., Docter, D., Stauber, R.H., Secker, C., Schlaad, H., Weidner, S., Mohr, B., Maskos, M., Bertin, A. (2015). Temperature-triggered protein adsorption on polymer-coated nanoparticles in serum. Langmuir, 31(32), 8873–8881.

[30] Zheng, X., Zhang, C., Bai, L., Liu, S., Tan, L., Wang, Y. (2015). Antifouling property of monothiol-terminated bottle-brush poly(methylacrylic acid)-graft-poly(2-ethyl-2-oxazoline) copolymer on gold surfaces. J. Mater Chem. B, 3(9), 1921–1930.

AUTHORS BIOSKETCHES

Shima Mohammadi Ziari, Department of Pharmaceutical Chemistry, Shahryar Branch, Islamic Azad University, Shahryar, Iran

Gita Bagheri, Assistant Professor, Department of Chemical Engineering, Shahryar Branch, Islamic Azad University, Shahryar, Iran, *E-mail: bagheri.gita@yahoo.com*