The preparation of dual-functional hydrogel as the surface coating of plastics in biomedical applications

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ABSTRACT

Poly(2-ethyl-2-oxazoline) (PEOXA) is among polymers that have been reported to show anti-adhesive (bio-passive) behavior in numerous biomaterial applications. This work aimed to develop dual-functional hydrogel coatings, with PEOXA as hydrophilic polymer and benzophenone as cross-linker. PEOXA was partially hydrolyzed in acidic conditions to form PEOXA-m% EI that contains ethyleneimine (EI) groups. The EI groups were used as conjugation sites for incorporation of benzophenone (BP) molecules to PEOXA chains to form PEOXA-m% EIBP. Thin films of surface-attached polymer networks were generated from PEOXA-m% EIBP copolymers composed of PEOXA as platform and benzophenone as crosslinker units. The polymer became crosslinked through benzophenone units and forms a hydrogel as biopassive platform during irradiation with ultraviolet (UV) light. Laminin was incorporated into biopassive polymer network to allow for preparation of dual-functional hydrogel. NMR spectra indicated successful control of PEOXA partial hydrolysis and conjugation of benzophenone molecules to PEOXA chains. Ellipsometry and ATR-FTIR results showed that wavelength of UV light during C, H-insertion reaction influences stability of polymer network (hydrogel) on the substrate surface. XPS spectra verified that a stable film could be generated using suitable UV light during preparation of polymer network. Cell culture study on laminin/PEOXA-coated PMAA plastics showed dual-functional properties.

Keywords: biomedical, hydrogel, photo-crosslinker, plastics, poly(2-ethyl-2-oxazoline).

INTRODUCTION

Biomaterials, including biomedical devices, are materials in contact with living tissues and/or biological fluids (blood, urine, saliva, tears, etc.), intended to diagnose, replace, treat, or enhance the performance of particular organs/functions in the body. Examples of biomaterials are hip implants, urinary catheters, teeth implants, contact lenses, bio-sensors, and various surgical and medical devices. The more detailed description and classification of biomaterials are available in a review by Chen and Thouas (2015). Globally, there is an increasing demand for biomedical devices as life quality and life expectancy increase in this modern society. Both clinical demand and patient expectations will continue to grow (Holzapfel *et al.*, 2013).

Despite their expected roles in increasing the life quality of patients, the contact of biomedical devices with a human body very often, if not always, trigger the fear of non-specific protein adsorption, bacterial infections, and foreign body response, in which the foreign objects with their artificial surfaces are not recognized by the mammalian cells, and thus treated as “stranger(s)” that must be removed/eliminated from the body (Castner & Ratner, 2002). It was previously believed that any

biomedical devices should present a biopassive surface, i.e., a surface that is bioinert, repellant to any biological entities and microorganisms to minimize the foreign body response. However, recent findings suggest that interactive, biomimetic surfaces are indeed required for enhancing biomaterials performance (Bellis, 2011).

It is now understood that implanted materials should not only provide structural support for damaged tissues, but also integrate with these tissues, and ideally, promote regeneration. Although the mechanisms are not yet well-understood, many researchers in the field of biomaterials are now striving to modify artificial surfaces with biologic elements to promote integration with surrounding tissues (Bellis, 2011). Thus, a scaffold that prevents non-specific interactions with non-targeted entities (biopassive/non-fouling/anti-adhesive) but enhances specific receptor-mediated cellular responses (bioactive) is in increasing demand in the field of tissue engineering.

The combination of bioactive moieties and the biopassive platform results in the so-called dual-functional surface coatings. In the context of tissue engineering, studies on dual-functional surface coatings have been previously reported (Chen *et al.*, 2015; Loschonsky *et al.*, 2008; Subbiahdoss *et al.*, 2009; Subbiahdoss *et al.*,

2010). In particular, we have previously reported a dual-functional hydrogel from combined poly(N, N-dimethyl methacrylate) (PDMAA) and laminin (Pidhatika *et al.*, 2019b).

The presented work aimed at developing dual-functional hydrogel coatings, in which poly(2-ethyl-2-oxazoline) (PEOXA) (Pidhatika *et al.* 2019a) was chosen as the hydrophilic polymer and benzophenone were chosen as the cross-linker. Laminin was then covalently grafted to the hydrophilic (and biopassive) polymer network as the bioactive moiety. Finally, MCF-7 cells were cultured on the dual-functional films. Since numerous biomedical devices are made of plastics (Czuba, 2014; Padsalgikar, 2017; Sastri, 2014a; Sastri, 2014b; Sastri, 2014c; Sastri, 2014d), the dual-functional coating was studied on poly(methylmethacrylate) (PMMA) slide substrates. The reasons behind the selection of PEOXA as the biopassive platform, and laminin as the bioactive compounds are available in our previous publications (Pidhatika *et al.*, 2019a; Pidhatika *et al.*, 2019b). For the crosslinker, benzophenone is classified as a non-specific cross-linker that can bind to any C-H bonds in its vicinity. This property of benzophenone offers a simple and effective process to covalently attach polymer layers to various solid surfaces using photo-irradiation method, as reported by Prucker *et al.*, (1999). Dahm *et al.* (2001) used benzophenone monolayer to covalently bind various hydrophilic polymers onto glass slide surfaces. Not only for surface attachment, benzophenone was also used to fabricate (surface-attached) hydrogels, as reported by Murata *et al.* (2004). The kinetics of the generation of surface-attached polymer networks containing benzophenone cross-linker units through C-H insertion reactions has been studied systematically (Körner *et al.*, 2016). The success story of benzophenone as a photo reactive cross-linker to fabricate as well as covalently deposit polymer networks simply and effectively for numerous, wide-ranging, applications is still continuously reported until very recently (Kleber *et al.*, 2017; Scherag *et al.*, 2017; Schuler *et al.*, 2016).

Different from our previous publication (Pidhatika *et al.*, 2019b) where PDMAA was used as the biopassive platform, the presented work focuses on PEOXA as the biopassive platform. Furthermore, different from our other previous publication (Pidhatika *et al.*, 2019a) where the potency of the PEOXA network as biopassive platform was evaluated, the presented work provides more details on the synthesis and bulk characterization of benzophenone-bearing PEOXA, as well as on surface characterization of the hydrogel. Moreover, the PEOXA biopassive network was combined with laminin as a bioactive compound.

Characterization of synthesized polymers was performed using nuclear magnetic resonance spectroscopy (NMR). Characterization of immobilized hydrogel on the surface was performed through ellipsometry, attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR), and X-ray Photoelectron Spectroscopy (XPS). Finally, the dual-functional hydrogel was prepared on PMMA plastics substrates, followed by

characterization using cell culture and optical microscopy. A similar polymer has been reported by other researchers (Dhende *et al.*, 2011; Lambermont-Thijs *et al.*, 2010; Li *et al.*, 2019). However, to the best of our knowledge, none of their reports provided the details as highlighted in this report.

MATERIALS AND METHODS

Materials

Poly(2-ethyl-2-oxazoline) (PEOXA) 50 kDa, 4-(bromomethyl) benzophenone, organic solvents, and physiological buffer were purchased from Sigma-Aldrich. MCF-7 cells were provided by the Laboratory for Bioinformatics and Molecular Genetics, Biology Department, University of Freiburg. Triethoxy-benzophenone silane was synthesized from triethoxysilane and 4-allyloxybenzophenone, and provided by the Laboratory for Chemistry and Physics of Interfaces. Silicon wafer and poly(methylmethacrylate) (PMAA) substrates were purchased from Thermo Fisher Scientific.

Methods

All synthesized polymers were characterized using ¹H-Nuclear Magnetic Resonance Spectroscopy (¹H-NMR). Prior to immobilization, crosslinking, and characterization on plastics (PMMA) surfaces, the formation of polymer network and its performance were investigated on modified silicon wafers. X-ray Photoelectron Spectroscopy (XPS) enabled the determination of chemical composition. Ellipsometry and Atomic Force Microscopy (AFM) enabled the determination of hydrogel film thickness, gel content, and stability. Cell culture study and phase-contrast microscopy on hydrogel-coated plastics substrates determines the aimed dual-functional properties of the hydrogel. The following protocols were adapted from previous reports (Pidhatika *et al.*, 2019a; Pidhatika *et al.*, 2019b).

Preparation of silicon wafer substrate with C-H bonds on its surface

PEOXA-11% EIBP was dissolved in ethanol (50 or 100 mg/ml) and filtered through a 0.45 µm filter. The polymer solution stock was stored at 4 °C and pre-warmed up to room temperature before use. Silicon wafer was cut (typically 1.5x1.5 cm) and cleaned with isopropanol, toluene, ethanol, and acetone. After drying with nitrogen, triethoxy-benzophenone silane was spin-coated (600 rpm, 100 s) on top of the wafer. The wafer was then heated up to 120 °C on a hot plate for 30 min. After cooling down to room temperature, the wafer was thoroughly rinsed with toluene to remove un-attached triethoxy-benzophenone silane, followed by blow-drying under Nitrogen.

Hydrogel formation, immobilization, and stability

Hydrogel formation, immobilization, and stability study were performed following the previously published protocol (Pidhatika *et al.*, 2019a). The polymer solution was spin-coated on top of the above-described wafer. The

polymer was irradiated under UV light in a Stratalinker® UV crosslinker, followed by rinsing with dissolving solvent and blow-drying under nitrogen. The wafer was then incubated in a physiological buffer (in this case PBS was used). After 24 h, the wafer was thoroughly washed with aquadest and ethanol, followed by blow-drying under nitrogen. The film thickness was measured using ellipsometry (*thickness* t_1). The prolonged incubation time of the wafer in the buffer, up to 30 days, was applied to investigate the hydrogel stability. The film thickness was measured from time to time, resulting in *thickness* t_n , where n is 1, 2, 3, ..., 30. The remaining thickness was calculated according to equation 1. The chemical characterization was performed by means of ATR-FTIR and XPS spectroscopy.

$$\text{Remaining thickness} = \frac{t_n}{t_1} \times 100\% \quad (1)$$

Cell culture study

Cell culture study was performed following the previously published protocol (Pidhatika *et al.*, 2019b). PEOXA-11% EIBP polymer solution with a concentration of 50 mg/ml in ethanol was spin-coated (600 rpm, 100 s) onto a PMMA slide. The slide was placed inside a petri dish after spin-coating. After pre-irradiation of PEOXA-11% EIBP with UV light at 365 nm, the petri dish was taken out of the UV crosslinker (Stratalinker®). Laminin was drop-coated on top of the polymer layer and the crosslinking process was continued. To keep the hydrogel sterile, antibacterial sodium azide was added to PBS buffer that will be used to incubate the hydrogel. The concentration of sodium azide in the PBS was 0.1% v/v. Then, 3 ml of the PBS was placed inside the petri dish and left for 24 h. Next, the petri dish was twice rinsed and thoroughly washed with pure PBS to remove traces

of sodium azide (possibly toxic to cells). MCF-7 cells in a cell medium were then cultured on the modified-PS surface inside the petri dish. After 24 h of cell culture on the modified-surfaces, the cell adhesion was observed using phase-contrast microscopy.

RESULTS AND DISCUSSION

The presented work focused on the preparation of dual-functional hydrogel as coating on plastics (polymeric) surfaces for biomedical applications. Figure 1 illustrates the strategy to achieve the objective. In this work, PMMA was used as the plastics substrates, benzophenone as the photocrosslinker, PEOXA as the hydrophilic (biopassive) polymers, and laminin as the cell adhesive (bioactive) molecules.

Synthesis and Characterization of Poly(2-ethyl-2-oxazoline)-m% ethyleneimine

Poly(2-ethyl-2-oxazoline)-m% ethyleneimine (PEOXA-m% EI) was synthesized by partially hydrolyzing PEOXA in acidic conditions, following previously published protocol (Figure 2) (Dhende *et al.*, 2011; Lambermont-Thijs *et al.*, 2010).

The characterization of the resulting PEOXA-m% EI was performed using Nuclear Magnetic Resonance spectroscopy (NMR). Figure 3, 4a, and 4b show the ¹H-NMR spectrum acquired from PEOXA, PEOXA-6% EI, and PEOXA-80% EI, respectively. The decrease of the peak a and b also the increase of peak d in the spectra when comparing the three Figures (3, 4a, and 4b) demonstrated the increase of hydrolysis degree of the PEOXA chain. Figure 5 shows the degree of hydrolysis as a function of reaction time, as obtained in this study. As expected, the degree of hydrolysis increased with an increase in reaction time.

Figure 1. The strategy on the preparation of dual-functional hydrogel on plastics (polymeric) surfaces (Pidhatika *et al.*, 2019b).

Synthesis and Characterization of Poly(2-ethyl-2-oxazoline)-m% Ethylene Imine Benzophenone

The 4-(bromomethyl) benzophenone was then grated to either PEOXA-6% EI or PEOXA-11% EI, using the protocol reported by Dhende *et al.* (2011). The synthesis route is depicted in Figure 6, and it was performed in tertiary amyl alcohol.

The characterization of the resulting poly(2-ethyl-2-oxazoline)-m% ethylene imine benzophenone (PEOXA-m% EIBP) was performed using Nuclear Magnetic Resonance spectroscopy (NMR). Figures 7a and 7b show the ^1H -NMR spectrum acquired from PEOXA-6% EIBP and PEOXA-11% EIBP, respectively. As expected, peaks d, e, and f (relative to peak c) in Figure 7b show higher intensities compared to those in Figure 7a, due to the higher amount of EIBP content in PEOXA-11%EIBP.

In addition to ^1H -NMR, ^{13}C -NMR was performed to PEOXA-11%EIBP. The resulting ^{13}C -NMR spectrum of PEOXA-11% EIBP was then compared to that of PEOXA-11% EI (Figure 8a and b). In addition to peaks a, b, c, d, and g in Figure 8a, the ^{13}C peaks e, f, and h originated from the methyl benzophenone group were detected in Figure 8b. Combined with the ^1H -NMR spectra, the ^{13}C -NMR spectra demonstrate the success of the benzophenone grafting to the PEOXA-11% EI chains.

Immobilization of the Benzophenone-functionalized PEOXA on the Surface, Hydrogel Formation, and Stability

After systematic study and validation of successful polymer synthesis using ^1H - and ^{13}C -NMR characterization method, PEOXA-11% EIBP was used to modify the surface of plastics material (in this case PMMA), according to the strategy as depicted in Figure 1. Upon irradiation under UV light, the benzophenone groups react according to C, H-insertion mechanism (Körner *et al.*, 2016), as illustrated in Figure 1, leading to crosslinking and polymer network (hydrogel) formation on the substrate surface. Simultaneously with polymer network formation, covalent immobilization took place, provided that C-H bonds are available on the substrate surface.

Since PMMA is an organic compound, abundant C-H bonds are available on its surface. However, the methods (ellipsometry for film thickness measurements and FTIR for functional group detections) that can be used to characterize the properties of surface-attached hydrogel require substrate properties that cannot be provided by plastics materials. Thus, before immobilization to PMMA surface for validation of dual-functional properties, the PEOXA-11% EIBP polymer was immobilized on silicon wafers that were modified with an ultrathin film of organic compound (triethoxy-benzophenone silane) that contains

Note:

(1): Poly(2-ethyl-2-oxazoline): PEOXA

(2): Poly(2-ethyl-2-oxazoline)-m%poly(ethyleneimine): PEOXA-m% EI

Figure 2. The acidic partial hydrolysis of PEOXA to PEOXA-m% EI.

Figure 3. The typical ^1H -NMR spectrum of PEOXA.

sufficient C-H bonds like plastics surfaces. This strategy allowed for investigation on the initial and remaining thickness of hydrogel film after certain incubation time in

relevant medium, i.e., determination of film stability, using ellipsometry method that requires reflecting substrates (McCrackin *et al.*, 2001).

Figure 4. The typical ^1H -NMR spectrum of a) PEOXA-6% EI, and b) PEOXA-80% EI.

Figure 5. Degree of hydrolysis of PEOXA as a function of time, in acidic condition, with an excess amount of HCl relative to the amide concentration.

(dry) film thickness was measured from time to time using ellipsometry, and the remaining thickness was calculated relative to the layer thickness after 1 (one)-day exposure time. The results from the stability test are shown in Figure 9.

$$\begin{array}{ccc} \left[\text{N} \begin{array}{c} \text{H}_2\text{C} \\ \text{CH}_3 \end{array} \text{---} \text{C} \begin{array}{c} \text{H}_2 \\ \text{H}_2 \end{array} \right]_x \left[\text{N} \text{---} \text{C} \begin{array}{c} \text{H}_2 \\ \text{H}_2 \end{array} \right]_y & \xrightarrow{\text{K}_2\text{CO}_3} & \left[\text{N} \begin{array}{c} \text{H}_2\text{C} \\ \text{CH}_3 \end{array} \text{---} \text{C} \begin{array}{c} \text{H}_2 \\ \text{H}_2 \end{array} \right]_x \left[\text{N} \begin{array}{c} \text{CH}_2 \\ \text{C} \begin{array}{c} \text{C}_6\text{H}_5 \\ \text{C}_6\text{H}_4 \end{array} \end{array} \right]_y \\ \text{(1)} & \text{(2)} & \text{(3)} \end{array}$$

(3): Poly(2-ethyl-2-oxazoline)-m% ethyleneiminebenzophenone (PEOXA-m% EIBP)

Chemical structure of poly(2,2'-(1,4-phenylene)-5,5'-bibenzimidazole) is shown with proton assignments: **a** (CH₃), **b** (CH₂), **c** (NH), **d** (NH), **e** (CH₂), and **f** (aromatic protons). The ¹H NMR spectrum in CDCl₃ shows peaks corresponding to these assignments, with additional peaks for solvent (CDCl₃, CH₂Cl₂) and water (H₂O). The aromatic region shows a multiplet labeled **f** and **f'**.

The figure displays the ^1H NMR spectrum of poly(2,2-bis(4-phenyl-1,3-oxazol-5-yl)propane) in CDCl_3 . The chemical structure of the polymer is shown as an inset, with protons labeled a through f. The spectrum features several peaks: a triplet at ~7.2 ppm (f, f'), a singlet at ~7.0 ppm (f, f'), a broad peak at ~3.5 ppm (c), a small peak at ~3.2 ppm (d), a peak at ~2.5 ppm (b), and a multiplet at ~1.2 ppm (a). Solvent peaks for CDCl_3 (~7.2 ppm) and CH_2Cl_2 (~3.5 ppm) are also present. The x-axis is labeled PPM and ranges from 0 to 8.

Figure 7. The typical ^1H -NMR spectrum of a) PEOXA-6% EIBP and b) PEOXA-11% EIBP (Pidhatika *et al.*, 2019a).

nm. The initial film thickness was approximately 700 nm. It is worthwhile to note that UV light irradiation energy increases with a decrease in its wavelength. In agreement with our previous finding, UV light at lower wavelength accelerates polymer degradation due to its too high energy (Pidhatika *et al.*, 2019a).

In addition to ellipsometry method that measures film thickness, attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) measurements were performed in attempts to probe changes of the chemical structure of the polymer during crosslinking reactions and network formation and to gain understanding on the

Figure 8. The typical ^{13}C -NMR spectrum of a) PEOXA-11% EI compared to b) PEOXA-11% EIBP.

Figure 9. The remaining thickness of PEOXA-11% EIBP film as a function of exposure time to the PBS buffer for a different wavelength of UV light during crosslinking reaction.

reason of hydrogel instability under UV light irradiation at a lower wavelength. Figure 10 shows the change of FTIR spectra of the spin-coated PEOXA-11% EIBP layer on a modified silicon wafer during UV light irradiation at 254 nm and 365 nm.

It was seen that UV light irradiation at 254 nm led to more extreme changes (at wavenumber around 3000-3500 cm^{-1} and 1500-1750 cm^{-1}) of the FTIR spectra of the PEOXA-11% EIBP film, which means more changes in the chemical structure and functional groups of the film. On the other hand, only slight changes were observed in the spectra of the layer upon UV irradiation at 365 nm, even up to 16.4 J/cm^2 of the irradiation energy. This phenomenon indicated that UV light at 254 nm is significantly more powerful to activate the benzophenone than at 365 nm, but might also be more destructive to the polymer as a whole. The latter was evident from the stability measurements (discussed in the earlier section), that UV light irradiation at 254 nm resulted in significantly lower hydrogel stability. Interestingly, no significant changes were observed in both PEOXA and PEOXA-11% EI spectra upon UV light irradiation at 254 nm and 365 nm respectively. Based on this finding, UV light at 365 nm was used for the hydrogel preparation on plastics surfaces.

The stability of hydrogel film on the surface when

prepared using suitable condition was further validated using another surface characterization method, i.e., X-ray Photoelectron Spectroscopy (XPS). For XPS measurements, PEOXA-11% EIBP was spin-coated on silicon wafers as XPS substrate modified with the ultrathin film of organic compound that contained sufficient C-H bonds like plastics surfaces. According to the strategy as illustrated in Figure 1, irradiation of the film with UV light at 365 nm should form a polymer network (hydrogel film) that strongly attached to the surface. XPS measurements were then performed on the following surfaces, i.e., 1) bare XPS substrate (silicon wafer), 2) spin-coated PEOXA-11% EIBP film, and 3) spin-coated PEOXA-11% EIBP film after UV light irradiation at 365 nm followed by incubation in physiological (PBS) buffer for 14 days.

While the survey XPS spectra of these surfaces have been reported in our previous publication (Pidhatika *et al.*, 2019a), here we present the high resolution XPS spectra. Figure 11 shows the deconvolution of high resolution C 1s, N 1s, and O 1s XPS spectra of the three above-mentioned surfaces. The bare silicon wafer substrate (Figure 11(a)-(c)) shows the typical carbon contamination (C aliphatic, C-O, and O-C=O) and an oxygen signal originating from the silicon oxide (Si-O), while nitrogen-containing species was absent. Spin-coating of PEOXA-11% EIBP on top of

Figure 10. FTIR spectra of a) PEOXA (above) and PEOXA-11% EIBP (below) upon different UV light irradiation dose at 254 nm, and b) PEOXA-11% EI (above) and PEOXA-11% EIBP (below) upon different UV light irradiation dose at 365 nm.

the (silanized) silicon wafer resulted in the domination of the spectra by PEOXA repeating units (Figure 11(d)-(f)).

Most prominently, a strong nitrogen signal due to the amide bonds (C-N) was observed. Correspondingly, the deconvolution of C 1s peak resulted in 3 (three) peaks, with 4 (four) components (C-C=C, H₃C-C, C-C-N, O=C). Only 1 (one) oxygen signal C=O was observed. Irradiation of the spin-coated PEOXA-11% EIBP (Figure 11(g)-(i)) resulted in the decrease of O=C and increase of C-C-C signal under the C 1s peak. In addition, the second oxygen species (C-OH) appeared. Both observed phenomena (in C 1s and O 1s) indicated the network formation of the polymer film due to the C, H-insertion reaction of benzophenone groups. Importantly, no significant changes in N/C signal intensity ratio between the two latter surfaces, which indicated the hydrogel film stability upon exposure to physiological buffer for 14 days.

Immobilization of Cell culture on Dual-functional PEOXA-based Hydrogel on PMMA Substrate

After systematic study and validation of hydrogel immobilization, formation, and stability, the PEOXA-

11% EIBP polymer was coated on PMMA substrate. In addition to PEOXA-11% EIBP that should play role as biopassive platform, cell adhesive molecule was added to the film to provide bioactivity. The combined biopassive and bioactive properties should result in dual-functional hydrogel as coating on plastics surfaces for biomedical applications. The strategy (with 2 (two)-step UV light irradiation) was previously illustrated in Figure 1. Briefly, to obtain a dual-functional surface, cell adhesive locations need to be generated on the surfaces. To achieve this, the photoreaction during hydrogel generation was stopped before complete conversion of the photoactive groups. Onto this layer, laminin as a well-known cell adhesion protein was drop-coated and the photo activation process continued, led to formation of a layer of covalently bound laminin. Figure 12 shows the microscopy images of laminin - PEOXA-11% EIBP (inside the circled area) - and PEOXA-11% EIBP (outside the circled area) - coated surfaces, after exposure to MCF-7 cells.

The films were prepared using different UV light irradiation dose, after exposure to MCF-7 cells for 24 h. The laminin (1 mg/ml in PBS buffer, 2 µl) was drop-coated

Figure 11. The high resolution C 1s, N 1s, and O 1s XPS spectra of (a-c) bare silicon wafer, (d-f) PEOXA-11%EIBP after spin-coating, and (g-i) PEOXA-11%EIBP after UV light irradiation at 365 nm followed by exposure to physiological (PBS) buffer.

Figure 12. The (a & c) 2x, and (b & d) 10x-objective microscopic images of laminin-decorated PEOXA hydrogel films on PMMA plastics substrates.

and allowed to dry after pre-irradiation of PEOXA film UV light (365 nm) at (a & b) 5 J/cm² and (c & d) 0 J/cm². Additional irradiation was then performed until the total energy dose for each surface reached 18 J/cm². The white solid circles were guides to the eye and represent laminin-decorated PEOXA areas. The 10x-objective microscopic images were taken inside the circled areas.

It is seen in Figure 12 that the cells attached preferably to laminin-containing area (inside the circled area). Although cell attachment was also observed on the biopassive background outside the circled area, the cells were significantly more concentrated on the bioactive sites. Comparing Figure 12 a) and c), more cells attached on the laminin-containing area that was irradiated with higher UV light energy dose, i.e., more cells on image (c) compared to those on image (a). The reason for this observed phenomenon is that no pre-irradiation (0 J/cm²) process to PEOXA film led to more active benzophenone crosslinker groups available for the covalent grafting of more laminin molecules on surface (c). Compared to surface (a), the PEOXA film was pre-irradiated (5 J/cm²) before the drop-coating of laminin, thus less active benzophenone crosslinker groups were available for the covalent grafting of laminin molecules. Moreover, image (d) shows better cell growth compared to image (b), indicative of more flattened cell morphology. Round and small cells as shown in image b) indicated failure of cell attachment and growth on the surface.

The observed phenomenon was in agreement with literature (Loschonsky *et al.*, 2008), which the authors reported increase of cell-adhesion with increasing amount of cell-adhesive molecules (RGD) on the surface, after incubation of the dual-functional surface in cell culture medium for 24 h. The result was, somehow different, with our previous publication where PDMAA network was used as the biopassive platform (Pidhatika *et al.*, 2019b). However, different polymers as biopassive coating were used. Furthermore, surface incubation in cell culture medium longer than 24 h has not been performed in this study.

CONCLUSION

The goal of the presented work was to build surface attached hydrogel layers on biomedical plastics that show dual-functional properties, i.e., exhibited a background having strongly cell-repellent properties and could exhibit in selected areas cell attractive properties. Poly(2-ethyl-2-oxazoline) (PEOXA) with desired content of ethyleneimine benzophenone (EIBP) groups was used for substrate modification. The benzophenone units acted as photocrosslinkers to form PEOXA-based polymer networks that is covalently attached on the organic (plastics) substrate. When substrates that contained C-H bonds on their surfaces were coated with the polymer and irradiated with UV light, the photoactive groups were activated and the reaction with carbon-hydrogen bonds nearby was initiated through CH-insertion. Through this procedure, surface-attached hydrogel layers were generated. Thus poly(methyl

methacrylate) (PMMA) substrates can be modified and transformed into biopassive platforms with this method. Concerning the layer formation, it was found that energy of the UV light was important for layer stability. When the wavelength was low (thus the energy was high), some degradation occurred and the stability decreased. To obtain a dual-functional surface, cell adhesive locations need to be generated on the surfaces. To achieve this, the photoreaction during hydrogel generation was terminated before complete conversion of the photoactive groups. Onto this layer laminin as a well-known cell adhesion protein was coated before continuing the photoactivation process. This step leads to formation of a layer of covalently bound laminin. The results from dual-functional test on the hydrogel-modified PMMA plastics substrate validated, 1) the role of benzophenone of the photocrosslinker, 2) the role of laminin as bioactive (in this context cell adhesive) compound, 3) the role of PEOXA hydrogel as biopassive platform, and 4) the strategy that was suitable for the modification of any biomedical plastics (provided C-H bonds are available) in tissue engineering applications.

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