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Synthesis and bioimmunological efficiency of poly(2-oxazolines) containing a free amino group

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Abstract Novel amphiphilic copolymers on the basis of 2-oxazolines containing a free amino group were prepared. The copolymers were synthesized by the living cationic polymerization of 2-ethyl-2-oxazoline (ETOX) and 2-(4-aminophenyl)-2-oxazoline (APOX). The main goal of this work was the synthesis of water soluble polymer material with the defined number of functional groups necessary for the attachment of proteins and polysaccharides. A high concentration of free amino groups allows immobilization of various biosubstances, e.g. drugs, proteins or polysaccharides. Thermal properties have been studied with respect to the composition of the copolymers. Cytotoxicity and the bioimmunological efficiency of the selected copolymer were studied.

1 Introduction

2-Oxazolines represent promising compounds for the preparation of well-defined polymers. Polymers prepared from 2-alkyl-2-oxazolines with a shorter alkyl substituent are water-soluble materials and thermosensitive polymers with a possibility for the utilization in drug and gene delivery, membrane technology or (bio)catalysis [1].

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They can be prepared by a living cationic polymerization initiated by the various electrophilic species, for example alkyl halides, sulfonic esters, strong acids and others [2]. 2-Oxazoline monomers can be used for the preparation of polymers with a controlled size and topology. In this way, polymers with a complex structure, such as block- and graft-copolymers [3] and star- [4] or comb-like polymers can be prepared.

Amphiphilic polymers have been prepared in several different ways. The first possibility is the polymerization of 2-oxazolines containing polar substituents with a lipophilic comonomer. One example is the preparation of copolymers from 2-methyl- or 2-ethyl-2-oxazolines and 2-oxazolines bearing a longer alkyl chain [5], or the copolymerization of 2-ethyl-2-oxazoline with 2-phenyl-2-oxazoline [6]. The second type of amphiphilic polymer materials can be prepared by the block copolymerizations of 2-alkyl-2-oxazolines with other polymers such as polylactides or polycaprolactone [7]. Another approach considers the use of 2-oxazoline macromonomers bearing another polymerizable group, usually containing an unsaturated bond. The graft and block copolymers were prepared by this method [8].

Polymers containing functional groups represent an important part of polymer therapeutics, especially in the drug delivery systems. Polymers containing functional groups in the side chain can be prepared directly from the 2-oxazoline monomer containing the required group, or by a polymer analogous reaction of polymer precursors. Preparation of polymers containing amino [9], carboxyl [10], mercapto [11], and aldehyde [12] groups in the side chain was recently described. In all cases, monomers containing a protected functional group were used for the polymer synthesis. The protecting group was removed after the polymerization process was completed.

The cationic polymerization of 2-oxazoline monomers containing another free functional group in the molecule has not been considered to be suitable for the synthesis of materials with functional groups. This is because of the possibility of side and transfer reactions during a propagation step [13]. However, Culbertson described the polymerization of a monomer containing a free amino group [14].

Polymers suitable for utilization in living organisms have to fulfill the strict requirements of biocompatibility, cytotoxicity, biodegradability, and/or immunocompatibility [15]. In vitro tests are commonly used as a first step in the characterization of the biocompatibility of a tested material before in vivo testing. Two model systems are often used for the characterization of the polymer biocompatibility. The monocyte-derived macrophages are ancient and phylogenetically conserved cells, and represent the first line of the host's defense after the epithelial barrier, against the microbes that reach mucosal surfaces. Therefore, they are one of the most crucial cell components of the innate immune responses against the infectious microbial challenge [16, 17]. On the other side, fibroblasts are not only cells producing an extracellular matrix; they also play a crucial role in wound healing together with the macrophages [18]. Fibroblast cell lines are commonly used to screen the adverse effects of different materials [19] by the evaluation of cytotoxicity using MTT assay [20].

This work is focused on the preparation of a biocompatible polymer material suitable for biomedical applications by using the cationic copolymerization of a 2-oxazoline containing aromatic amino group. Cell viability determination and establishment of phagocytic and metabolic activity of macrophages were used to assess the cytotoxicity and/or immunosuppressive effects of the selected copolymer for possible application in drug delivery and immunology.

2 Experimental part

2.1 Materials

Methyl 4-aminobenzoate, 2-aminoethanol, and methyl *p*-nitrobenzenesulfonate (all from Sigma–Aldrich) were used without purification. *N*,*N*-Dimethylacetamide (Sigma–Aldrich) was dried over a phosphorous pentoxide and distilled under a reduced pressure. 2-Ethyl-2-oxazoline (ETOX) (Acros) was stirred over KOH for 48 h and distilled over CaH_2 under a reduced pressure.

Phosphate buffered saline (PBS) and Trypsine–EDTA (both from Sigma–Aldrich), 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Calbiochem), fluorescein diacetate (FDA) (Invitrogen); DMSO and propidium iodide (PI) (Fluka); DMF (Merck); hydroxyethidine (HE) (Polysciences) were used for cytotoxicity and bioimmunological assays. Poly(ethylene imine) (PEI) (Sigma–Aldrich) was purchased as 50 wt% aqueous solution, and poly(vinyl alcohol) (PVA) (Acros) was 75% hydrolyzed polymer with a number average molar mass M_n equal to 2000 g/mol.

2-(4-Aminophenyl)-2-oxazoline (APOX) was prepared from methyl 4-aminobenzoate according to a known procedure [21]. m.p. 160–162°C (ethanol, lit. 160–161°C [21]). ^{1}H NMR (DMSO-d_6): 3.84 (t, 2H, NCH_2), 4.28 (t, 2H, CH_2O), 5.68 (s, 2H, Ar-NH_2), 6.55 (d, 2H, ar), 7.52 (d, 2H, ar); ^{13}C NMR (DMSO-d_6): 54.15; 66.76; 112.97; 114.3; 129.26; 151.76; 163.44.

2.2 Copolymerizations

Copolymerizations were done in bulk at 120°C for 5 h using methyl *p*-nitrobenzenesulfonate as an initiator. In all cases, an ampoule was dried and degassed in three freeze-thaw cycles, and the polymerizations were done in an argon atmosphere. The theoretical degree of polymerization was calculated from the equation $DP_{theor} = ([M_1] + [M_2])/[I]$; and its value was in all cases equal to 100. Resulting polymers were dissolved in 2 ml *N*,*N*-dimethylacetamide, terminated with 0.5 M methanolic KOH for 2 h at room temperature, and precipitated in diethyl ether. Copolymerizations were done in the range of content of APOX within 3–100 mol%. Acronyms of all copolymers are listed in Table 1. For example, the acronym AEOX10 means the copolymer is prepared from 10 mol% APOX and 90 mol% ETOX.

Cationic polymerization of APOX was done in *N*,*N*-dimethylacetamide solution in a concentration of 3 mol/dm^3 for 5 h at 120°C. Poly(2-ethyl-2-oxazoline) (AEOX0) was prepared by solution polymerization in *N*,*N*-dimethylacetamide at 90°C for 24 h using methyl *p*-nitrobenzenesulfonate as an initiator. Polymers used for the cytotoxicity and phagocytosis assays were purified by dialysis on the Spectra/Por Dialysis membrane Nr.6/MWCO 1,000 (Spectrum Laboratories, Inc.) for 24 h. The results are listed in Table 1.

2.3 Analytical methods

Calorimetric measurements were performed using a Mettler-Toledo DSC 821^e differential scanning calorimeter. Indium was used for the calibration of temperature and the heat of fusion. The glass transition temperature (T_g) of the samples was evaluated from the second heating of samples in the temperature interval from 0 to 250°C (10°C/min) under a nitrogen atmosphere. The ^{1}H and ^{13}C NMR spectra

Table 1 The results of bulk copolymerization of 2-ethyl-2-oxazoline (ETOX) with 2-(4-aminophenyl)-2-oxazoline (APOX) at different ratios of comonomers at 120°C for 5 h

Acronym of copolymer	ETOX[a] (mol%)	APOX[a] (mol%)	Yield (%)	$[NH_2]_{theor}$[b] (mmol/g)	$[NH_2]$[c] (mmol/g)	M_n (g/mol)	PDI	T_g (°C)
AEOX0	100	0		–	–	16200	1.08	56
AEOX3	97	3	79	–	–	9700	3.76	59
AEOX5	95	5	87	0.489	0.162	4670	4.04	62
AEOX7	93	7	81	0.676	0.434	4740	4.37	64
AEOX10	90	10	98	0.948	0.311	10800	2.59	66
AEOX11	89	11	88	1.037	0.512	6900	4.31	71
AEOX12	88	12	97	1.125	0.623	7760	4.09	72
AEOX15	85	15	91	1.381	0.830	5880	4.11	73
AEOX30	70	30	87	–	–	8200	3.37	98
AEOX50	50	50	79	–	–	6360	3.37	112
AEOX70	30	70	85	–	–	5340	2.84	119
AEOX100	0	100	93	–	–	7540	2.37	133

[a] ETOX, 2-ethyl-2-oxazoline; APOX, 2-(4-aminophenyl)-oxazoline

[b] $[NH_2]_{theor}$, the concentration of free amino groups calculated from the composition of the copolymers

[c] $[NH_2]$, the concentration of free amino groups determined by the colorimetric assay

of all compounds were recorded at room temperature on a Varian VXR-400 in DMSO-d_6 solutions.

Molar masses and polydispersity indices were determined by size exclusion chromatography (SEC) towards narrow distributed polystyrene standards (PSS, Mainz, Germany) using RI detector (Waters 2410) on 10 μm PSS GRAM precolumn and three 10 μm PSS GRAM columns with pore sizes 100, 1000 and 3000 Å. All measurements were done at 45°C with a flow rate of 0.8 ml min^{-1} controlled by toluene as a flow marker. *N*,*N*-dimethylacetamide + 0.1% LiBr was used as a mobile phase.

Content of free amino groups in the polymer samples was evaluated by the 2,4,6-trinitrobenzenesulfonic acid method using a 4-amino-*N*-(2-hydroxyethyl)benzamide as a reference [22]. The wavelength used was 342 nm. The results are expressed as moles of free amino groups per gram of polymer.

2.4 Cell lines and cultivation

Lymphoid mouse macrophage P388.D1 (Clone 3124) and rat fibroblast-like RAT-2 cell lines (both from ECACC) were used for the study of the bioimmunological assays or the cytotoxicity, respectively.

Cells were collected via a trypsinization with 0.05 M Trypsin-EDTA and counted in the Buerker chamber. As a growth media, a DMEM containing 5% fetal bovine serum (FBS), was used to culture a RAT-2 cell line, while RPMI with 20% horse serum was used for the growth of P388.D1 cells. Cells were incubated in the CO_2 incubator at 37°C, $c(CO_2) = 5\%$.

Cell culture preparations for the phagocytosis, oxidative burst, determination of free radicals, and cell viability measurements were done simultaneously as follows: Cells were harvested and counted by using the Buerker chamber. 1×10^5 cells were put into each well of a 12-well tissue culture plate and the respective amount of medium was added to the cells. Cells were incubated in the incubator at 37°C overnight in the presence of 5% of CO_2. The sample of untreated cells (control cells) and the cells with the AEOX10 solution were measured at the beginning of the experiment.

At the same time, the solutions of studied polymer (AEOX10) in the concentrations of 0.5 and 5 mg/ml, respectively, were added to the rest of the wells. Samples for the measurements of phagocytosis, oxidative burst, and viability staining were taken at four different times during the incubation (1, 3, 6, and 24 h).

2.5 Cytotoxicity assay

A MTT cytotoxicity assay was used for the estimation of cytotoxic properties of the studied polymers in the treatment of fibroblasts. Cells were collected via trypsinization and counted in the Buerker chamber. Cells were then seeded in the 12-well tissue culture plate at the concentration of 1×10^5 per well and incubated in the growth medium overnight in the CO_2 incubator at 37°C, $c(CO_2) = 5\%$.

Polymers were dissolved in 1 ml of DMEM growth medium and sterile filtered through the 0.22 μm filter. They were added to cells in concentrations of 5 mg/ml, and

incubated in the CO_2 incubator at 37°C, c(CO_2) = 5% for 24 and 48 h. A stock solution of MTT (5 mg/ml) was dissolved in PBS. The MTT solution was added to the fresh DMEM growth medium at a final concentration of 0.5 mg/ml. Afterwards, the MTT in the DMEM medium was added to the cells and they were incubated at 37°C for 2 h. The purple crystals of a formed insoluble formazane were dissolved in DMSO. Cell viability was determined by comparing the absorbance spectra of treated and untreated cells (negative control) at 570 nm.

Cell viability of macrophages after treatment with AEOX10 (concentrations 0.5 and 5 mg/ml) was estimated by a PI/FDA staining. Briefly: 50 µl of FDA (0.024 mg/ml of freshly prepared aqueous solution), and 50 µl of PI (0.02 mg/ml dissolved in distilled water) were added to 500 µl of cell suspension. After 20 min of staining, the suspension of macrophages was washed with a saline. The combined double fluorescence of FDA^+ (viable) and PI^+ cells (dying or dead cells) was evaluated by a flow cytometry (FL1 vs. FL3) using a Beckman-Coulter FC 500 flow cytometer (Beckman Coulter Inc., Fullerton, California, USA).

2.6 Simultaneous phagocytosis and oxidative burst

The double fluorescence of FITC-labelled ingested *Staphylococcus aureus* cells and their hydroxyethidine oxidation to ethidium bromide during the respiratory burst of macrophages were evaluated by the flow cytometry (FL1 vs. FL2), using the Beckman-Coulter FC 500 flow cytometer. The cytometer was equipped with a 488 nm argon laser and a 637 nm HeNe collinear laser, and is controlled by CXP software. For each sample, the fluorescence histogram of 10,000 cells was generated and analyzed. Gates were set around debris and intact cells on forward scatter *vs.* a side scatter dot plot.

Quantitative determination of macrophage phagocytosis (ingestion of bacteria) and the subsequent digestion are multistep and multifactorial. Measurement of the ingestion of bacteria took place under the controlled conditions using a fluorescein labelled opsonised *S. aureus* (SPA-FITC) (Molecular Probes, The Netherlands). Metabolic activity was determined via the oxidative-burst of the stimulated transformation of originally non-fluorescent hydroxyethidine (HE) onto ethidium, which intercalates into DNA and gives rise to red fluorescence (excitation of 488 nm). Aliquots of macrophages treated with AEOX10 solutions (concentrations of 0.5 mg/ml and 5 mg/ml) were incubated with HE (the concentration of 15.75 mg in 5 ml of DMF) for 15 min at 37°C. Following the treatment with SPA-FITC for the next 15 min at 37°C, the reaction was stopped by placing the samples on ice. The subsequent lysis was performed for 15 min with an ice-cold ACK lysis buffer (200 ml deionized water; 1.658 g NH_4Cl; 0.2 g $KHCO_3$; 7.4 mg Na_2EDTA; pH 7.2–7.4).

2.7 Quantitative determination of free radicals

The cell culture supernatants were assayed for the total content of free radicals after 1, 3, 6, and 24 h of cultivation. A reagent kit based on it's chlorophyllin (sodium–copper salt of chlorophyll) ability to transfer electrons due to its electron-rich double bond structure ("Free radicals", Sevapharma Ltd., Czech republic) was used. The quantification of free radicals is based on a calibration of Fe^{2+}/Fe^{3+} reaction expressed in mmol/l of Fe^{2+} ions.

2.8 Computational statistical analyses

Statistical comparison between experimental groups was performed using one-way ANOVA and post hoc Bonferroni's and Tukey's tests. The results were significant if the difference between the analyzed groups equalled or exceeded the 95% confidence level ($P < 0.05$). Statistics were performed with ORIGIN 7.5 PRO software (OriginLab Corporation).

3 Results and discussion

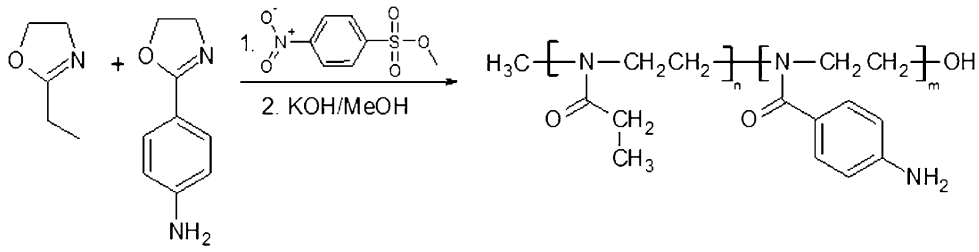
3.1 Copolymerizations

It is well known that the polymerization of simple 2-alkyl- and 2-aryl-2-oxazolines leads to the formation of poly (*N*-acyl- and *N*-aroylethylene imines). Among them, poly(2-ethyl-2-oxazoline) is the most recognizable as a biocompatible material and water soluble polymer with thermo-sensitive properties. Much less is known about the cationic polymerization of the monomers having a reactive group in the molecule. The cationic polymerization of 2-(4-aminophenyl)-2-oxazoline was studied by Culbertson and Xue [14]. The authors observed two different reaction modes. It was suggested that except of regular cationic polymerization, side reactions proceed with the participation of an amino group.

We used the copolymerization of 2-ethyl-2-oxazoline and 2-(4-aminophenyl)-2-oxazoline for the preparation of the polymer material containing free amino groups. It is expected that the copolymerization proceeds according to Scheme 1.

The copolymers were prepared in a good yield. The molar masses and polydispersity index (PDI) were determined by GPC. Molar masses of the copolymers ranged from 4500 to 11000 g/mol, which correspond to the theoretical values. However, the polydispersity index (PDI) had a much higher value than one that should correspond to the

Scheme 1 Copolymerizations of 2-ethyl-2-oxazoline (ETOX) with 2-(4-aminophenyl)-2-oxazoline (APOX)



pure ionic process. This also confirms that side reactions occur during copolymerization. Moreover, the polymerization of 2-ethyl-2-oxazoline is too fast to keep the control of cationic copolymerization and the resulted polydispersity is much higher than in the case of polymerization of 2-(4-aminophenyl-2-oxazolines). As seen in Table 1, values of PDI for copolymers with higher content of ETOX are higher than that ones for copolymers with higher content of APOX.

The content of free amino groups was estimated for the polymers soluble in water by the colorimetric assay of the complex with 2,4,6-trinitrobenzenesulfonic acid at 342 nm. The concentration of primary amino groups increased with the amount of APOX in the copolymer and corresponds to 35–65% of theoretical amount of amino group in the copolymer (Table 1).

The structure of the prepared copolymers was estimated by NMR spectroscopy. Figure 1 shows ^{1}H NMR spectra of APOX and one of the prepared copolymers, namely AEOX50. In the spectrum of the APOX, we can find a triplet of CH_2N at 3.84 ppm and a triplet of CH_2O at 4.24 ppm. Peak at 5.68 ppm can be attributed to the free amino group. Doublets of aromatic protons are located at 6.55 ppm and 7.52 ppm. The spectrum of AEOX50 reflects the formation of the copolymer, and hence contains signals of both building units. Broad singlets of ethylene imine protons of the main chain of both comonomers are located at 3.39 and 3.23 ppm. An ethyl group of ETOX contains two signals. The first signal, which was assigned to CH_3, is located at 0.96 ppm. The band of $–CH_2–$ is split into two broad signals at 2.06 and 2.30 ppm. The splitting of ethylene protons is typical for the ^{1}H NMR spectrum of poly (2-ethyl-2-oxazoline). The amino group was divided into two signals; the weaker signal is located at 5.42 ppm, and the stronger signal is located at 5.60 ppm. This means that only a part of NH_2 groups took part in the copolymerization. Signals of both aromatic protons are split into two doublets: 6.54 and 6.61 ppm for the first type of proton, and 7.56 and 7.64 ppm for the second type of aromatic proton. Small peaks at 8.19 and 7.85 ppm can be attributed to the amide groups of the side product.

Thermal properties of the prepared copolymers were measured by DSC (Fig. 2, second heating). In all cases, the only visible phase transition is the temperature of the glass transition (T_g) of the copolymer. Moreover, the existence of only one T_g is evidence that the copolymers are homogeneous materials. The temperatures of the glass transition are for all copolymers in the range from 56 to 133°C; the border values are the values for the homopolymers AEOX0 and AEOX100.

It was mentioned above that poly(2-ethyl-2-oxazoline) is soluble in water. The addition of the less polar APOX decreased the solubility of copolymers in water. We found that copolymers containing more than 15 mol% of APOX become insoluble in water and therefore are not suitable for the majority of medical applications. Good solubility of AEOX10 in water and sufficient amount of free amino group enables immobilization of a protein and polysaccharides on the copolymer. Therefore, the copolymer AEOX10 was selected from the series of the prepared copolymers for all types of cytotoxicity and bioimmunological tests. The results of the conjugations will be published elsewhere [23].

3.2 In vitro cytotoxicity studies

MTT test, a standard laboratory colorimetric assay for measurement of cellular growth, has been used for the evaluation of cytotoxicological properties of assessed (co)polymers [20].

In this study, a macrophage cell line P388.D1 (Clone 3124) and a fibroblast-like cell line RAT-2 were selected as model systems to characterize biocompatibility of polymers. RAT-2 fibroblasts were incubated with the AEOX10, PVA and PEI solutions, respectively, each in a concentration of 0.5 mg/ml. PVA is known as non-toxic [24]; in this study PVA was used as a positive control. On the other hand, PEI was shown to be highly toxic [25] and was therefore used as a negative control.

Cells were incubated with the above mentioned polymers for 24 and 48 h. As seen in Fig. 3, AEOX10 shows 100% viability after 48 h in agreement with PVA. The cell viability was decreased to 40% after 48 h in the presence of PEI. In conclusion, the AEOX10 causes no harm to cells under the conditions used and seems to be suitable as a non-toxic material for utilization in living organisms.

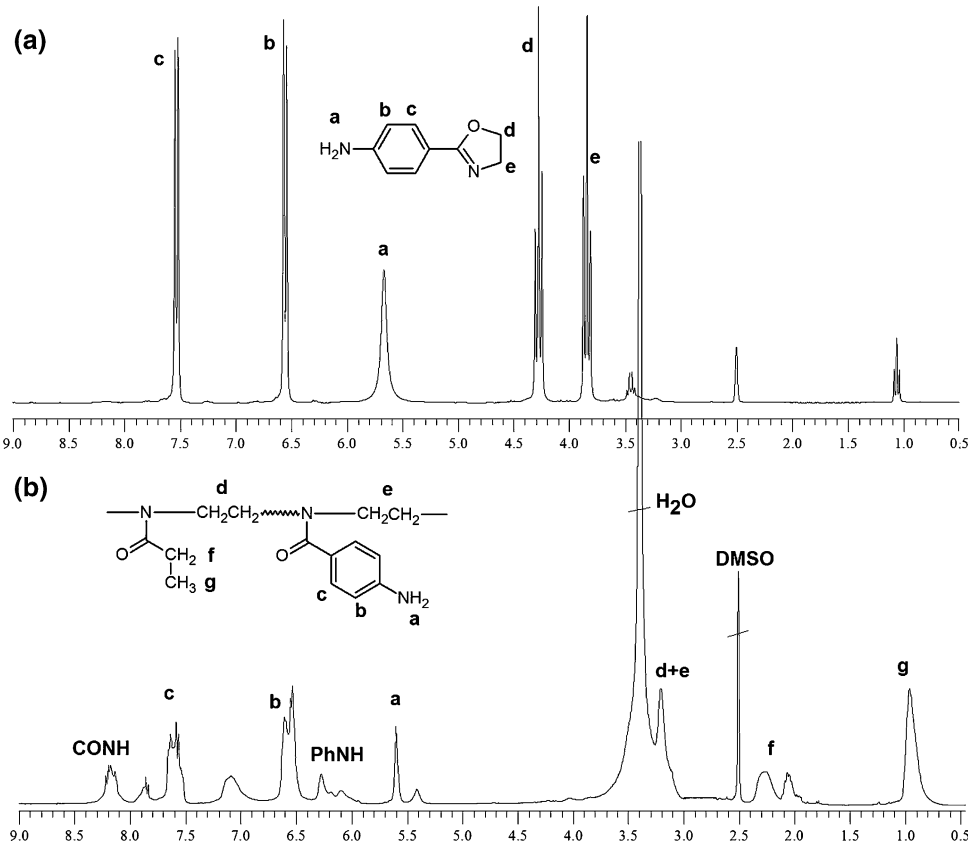


Fig. 1 ^{1}H NMR spectrum of APOX (**a**) and AEOX50 (**b**) measured in DMSO-d_6

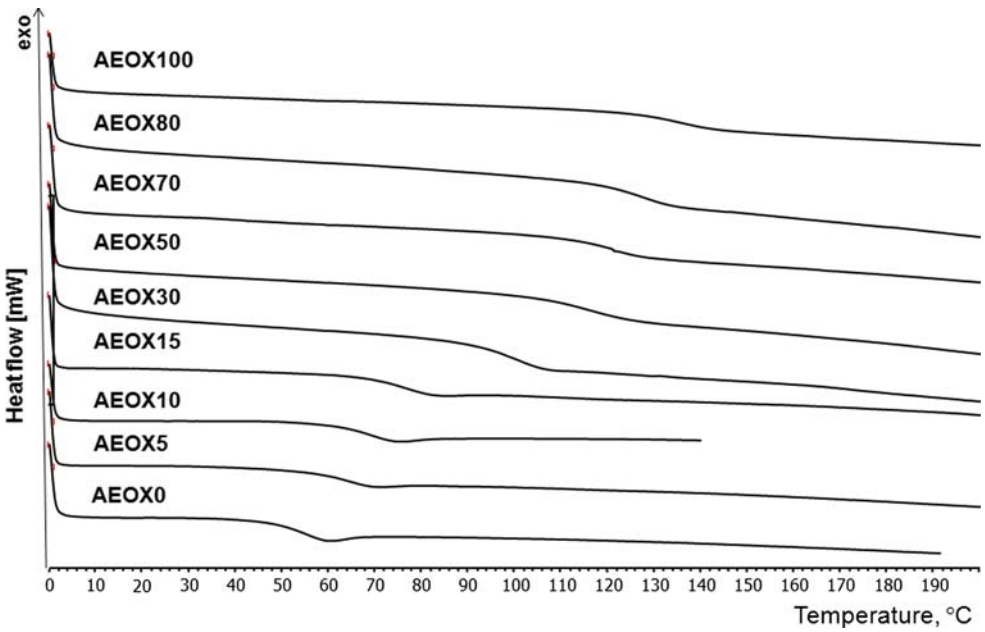


Fig. 2 DSC curves of copolymers with a different content of 2-(4-aminophenyl)-2-oxazoline (APOX)

3.3 Bioimmunological assays

Monocyte–derived macrophages are the representative cells of the host defense innate immunity arm against the microbes reaching surfaces of a mucosa [16, 17]. Macrophages are equipped with a limited number of pathogen recognition receptors (PRR) which recognize invading microbes via their pathogen-associated molecular patterns, i.e. the macromolecules that are characteristic for the microbial surfaces or are released by microbes in the process of infection (e.g. LPSs and teichoic acids, shared by gram-negative and gram-positive bacteria, respectively,

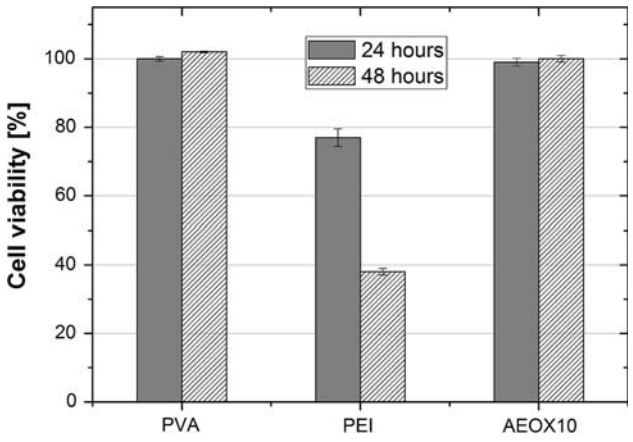


Fig. 3 Cell viability determined by MTT test after AEOX10 treatment compared with the positive (PVA) and the negative (PEI) controls. The treatment time was 24 and 48 h, respectively. The data are represented as mean ± SEM for triplicates

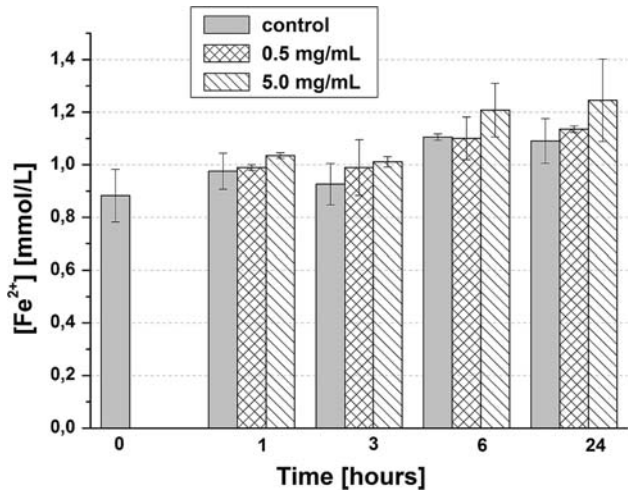


Fig. 4 Induced generation of free radicals by different concentrations of AEOX10. The quantification is expressed as a concentration of Fe^{2+} ions in mmol/l. The data are represented as mean ± SEM for duplicates

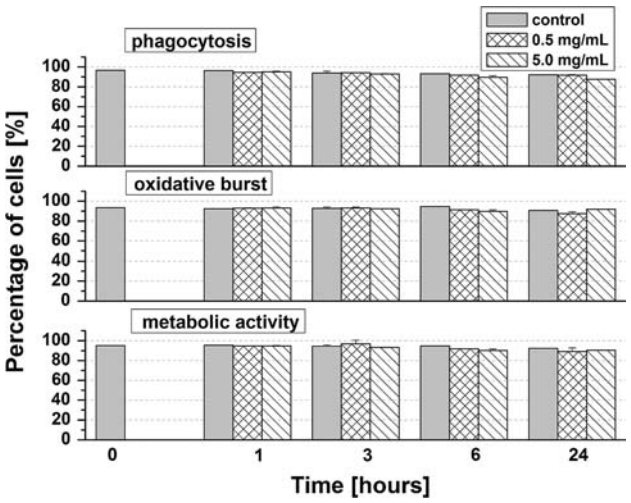


Fig. 5 Influence of.AEOX10 on phagocytic activity, oxidative burst and metabolic activity (phagocytosis and oxidative burst) of macrophage. Phagocytic activity, oxidative burst and metabolic activity are expressed as percentage of cells actually undergoing these processes toward total cell number equal to 10,000. The data are represented as mean ± SEM for duplicates

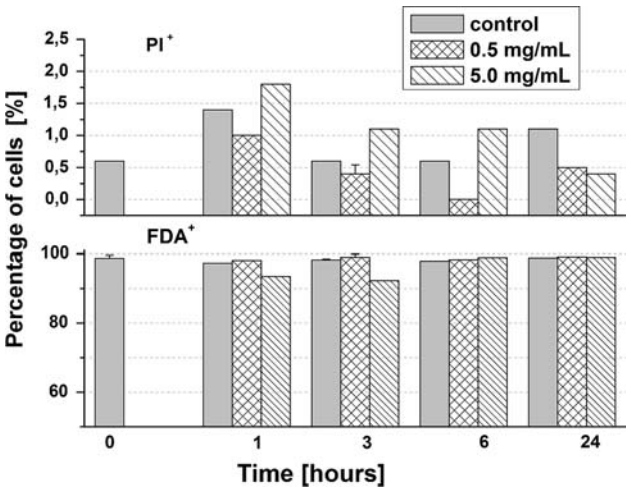


Fig. 6 Time and concentration dependent cell viability assay. Quantification of cell viability is expressed as percentage of PI^+ FDA^- or FDA^+ PI^- cells of total number 10,000 cells. The data are represented as mean ± SEM for duplicates

yeast cell wall mannan, etc.). The main functions of PRRs include opsonization, the activation of complement and coagulation cascades, phagocytosis, the induction of inflammatory cytokines, and the induction of apoptosis [26].

During the process of ingestion, macrophages generate large amounts of highly reactive molecules, predominantly oxygen radicals. The capability to generate and release reactive oxygen species is an exclusive attribute of the activated macrophage cells [27]. In our experiments, the time- and concentration-dependent release of free radicals (Fig. 4) was evaluated. AEOX10 treatment of macrophages did not result in a progressive generation of highly reactive oxygen species. Only a small, statistically non-significant increase of free radicals was observed similar to that in non-AEOX10-treated macrophage control cells. Evidently, both concentrations (0.5 and 5 mg/ml) of AEOX10 represent a safe dose and did not interfere with the functional properties of phagocytic cells.

Phagocytosis and metabolic activity remained without any undesirable immunosuppressive changes (Fig. 5). Moreover, these results are also confirmed by the cell viability tests (Fig. 6). Almost 100% macrophage FDA activity persisted for 24 h while either of the concentrations of AEOX10 was applied. Cell counts were

determined at the beginning of the experiment, after 6 h, and at the end of experiment. Based on these results we can conclude that the treatment with both doses of AEOX10 did not affect the normal cell cycle and the cell proliferation in comparison with the control.

4 Conclusion

Copolymers containing a free amino group were prepared by the cationic copolymerization of 2-ethyl-2-oxazoline and 2-(4-aminophenyl)-2-oxazoline. Although the cationic polymerization of 2-oxazolines belongs to the living polymerization processes, prepared copolymers displayed broader polydispersity than was expected. A possible explanation could be the side reaction of a free amino group of a monomer. The spectral analysis of free amino groups in the resulting polymers showed that polymers contain approximately half the amount of primary amino groups in comparison with the theoretical value. Cytotoxicity tests confirmed the high cell viability of fibroblasts and macrophages in the presence of the copolymer AEOX10 containing 10% of aromatic building units. Based on ex vivo immunological assays, we can conclude that the copolymer AEOX10, even in the concentration of 5 mg/ml, does not exert any immunosuppressive or toxic behaviour. The prepared copolymers represent biocompatible materials suitable for the immobilization of bioactive species, such as proteins, polysaccharides or drugs.

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