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On the biodegradability of polyethylene glycol, polypeptoids and poly(2-oxazoline)s

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ABSTRACT

Despite being the gold standard of hydrophilic biomaterials and well known sensitivity of polyethylene glycol (PEG) against oxidative degradation, very little information on the decomposition of PEG under biological oxidative stress can be found in the literature. Poly(2-oxazoline)s (POx) and polypeptoids (POI), two pseudo-polypeptides, have attracted some attention for the use as biomaterials and alternative to PEG with an altered stability against oxidative degradation. All three polymer families are supposedly non-biodegradable, which could be seen as one of their main disadvantages. Here, we present evidence that PEG, POx and POI are degradable by oxidative degradation under biologically relevant conditions. Transition metal catalysed generation of reactive oxygen species (ROS) leads to a pronounced time and concentration dependent degradation of all polymers investigated. While we do not envision oxidative degradation to be of relevance in the short-term usage of these polymers, mid- and long-term biodegradability *in vivo* appears feasible. Moreover, influence in ROS mediated signalling cascades may be one mechanism how synthetic polymers influence complex cellular processes.

1. Introduction

Many synthetic biomaterials, including the widely applied polyethylene glycol (PEG) and essentially all polymers derived from radical polymerization reactions comprising a C–C polymer backbone are considered non-biodegradable. However, stability of polymeric materials strongly depends on the applied conditions and important factors include water content, pH, temperature, exposure to UV light, presence of enzymes, oxygen or oxidants such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) [1,2]. For biodegradability, predominantly hydrolytic and proteolytic (enzymatic) degradation is typically discussed [3–5]. However, major metabolic pathways and (patho)physiological processes in many organisms, including humans, depend on, or are connected with oxidative modification or degradation of molecules [6–13]. Also, host-defence mechanisms mediated by immune cells such as neutrophils or monocytes/macrophages are connected with production of relatively large amounts of ROS. *In vivo*, ROS can be generated by single electron transfer to dioxygen, a process that this catalysed by a variety of enzymes, including P450 cytochromes, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), lipoxygenase, NADPH-oxidase (NOX) and dual oxidase (DUOX). Regardless of the source, transition of the generated ROS is mediated within the ROS cascade (Fig. 1). In recent years, polymeric biomaterials that are sensitive to oxidative stimulus have received considerable attention [14–20]. Almutairi et al. argued recently that "there are few if any polymeric systems able to undergo degradation and cargo release on encountering biologically relevant (50–100 μM) H_2O_2 concentrations." [18].

On the other hand, it is well understood that PEG is prone to (per)oxidation and resulting degradation of the polymer chain [22,23]. However, to the best of our knowledge, this has not been studied or discussed in a biologically meaningful context in solution, while degradation of surface tethered PEG has been studied before [24,25]. Another recent study investigated the "physiologically relevant oxidative degradation" of PEG-based hydrogels that contain oligo(L-proline) segments [26]. Interestingly, while the response of oligo(L-proline) to ROS/RNS has been studied, PEG is

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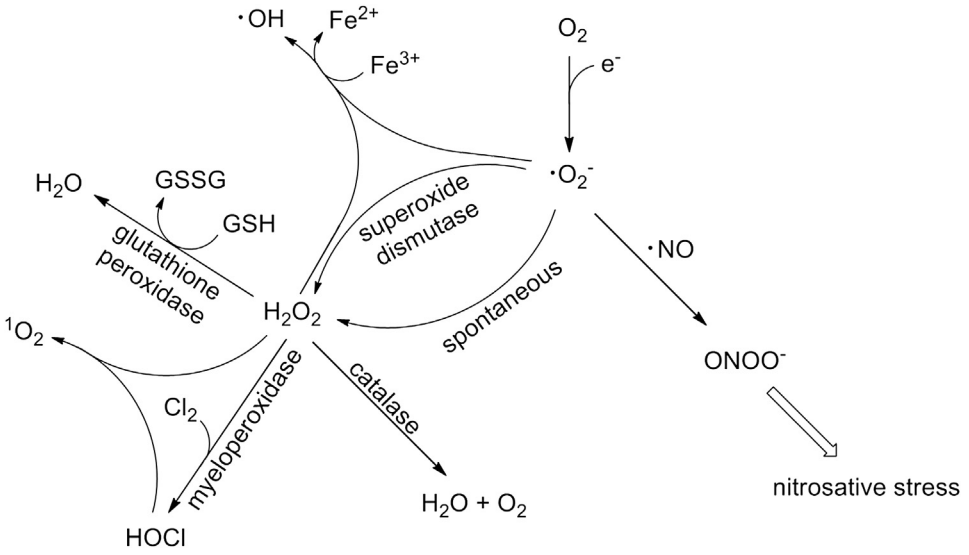


Fig. 1. Cascade of transitions between different reactive oxygen and nitrogen species, initiated by a single electron transfer to oxygen. Modified from Ref. [21].

assumed to be stable by the authors without experimental evidence [26]. Considering the known instability of PEG under such conditions, we hypothesized that this assumption may not be fully valid.

Oxidative degradation of proteins and (poly)peptides is well known to be important in their metabolism (Fig. 2) [27,28]. Besides secondary, oxidative degradation of tertiary amides in a biological context (rat liver microsomes) is also known [29]. We have investigated so-called pseudo-polypeptides based on the polymer families of poly(2-oxazoline)s (POx) [30–34] and N-substituted polyglycines (so-called polypeptoids, POI) [35–46] which have been discussed as chemically highly versatile and well-tolerated biomaterials and potential alternatives to PEG [47,48] (for recent reviews, see Refs. [30,49] and ref. [35]).

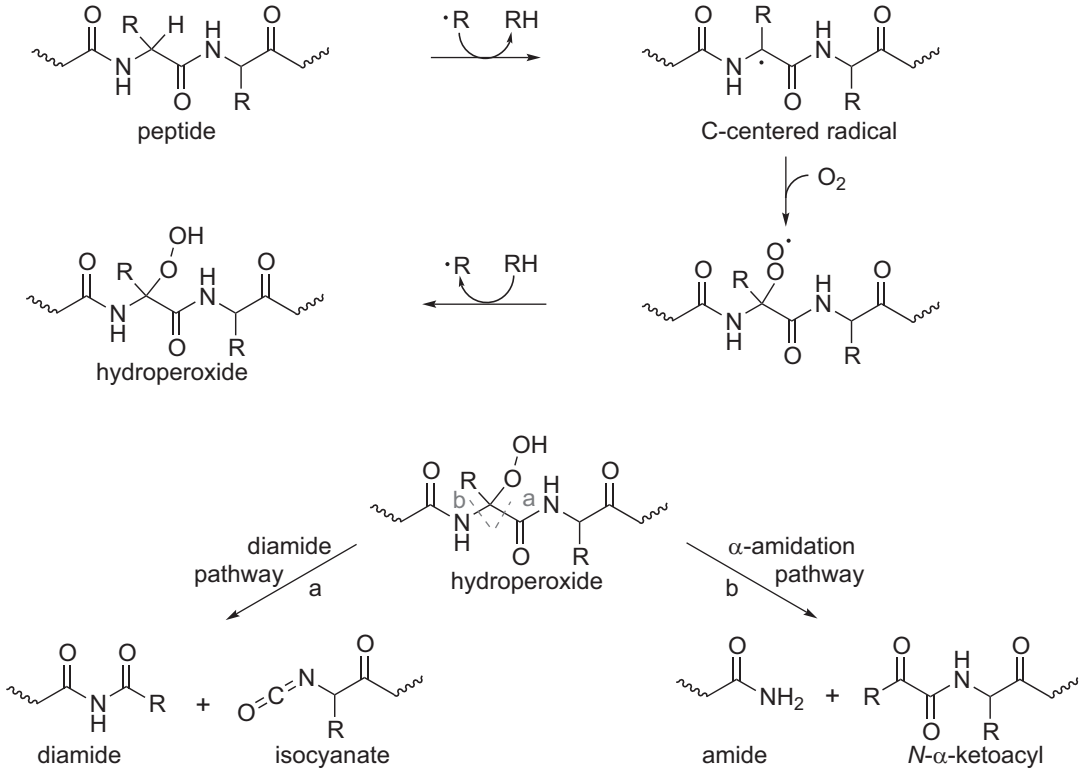


Fig. 2. Oxidative degradation of (poly)peptides. a) Degradation is initiated by H-abstraction at a C-α of the peptide followed by radical addition (e.g. dioxygen or •OH or •OOH). b) Exemplarily, formed hydroperoxides can undergo different degradations via the diamide pathways or the α-amidation pathway [28]. Both events lead to main chain scission.

Since pseudo-polypeptides bear a tertiary amide instead the secondary one, enzymatic degradation is severely hindered and the polymers are considered non-biodegradable. However, oxidative pathways (possibly mediated by enzymes) may represent the major mechanisms towards polymer biodegradation for this class of biomaterials. Textor and co-workers [25,50] and Veronese and co-workers [51] reported that POx, which are structurally related to poly(L-proline) (Scheme 1), are more stable against oxidative degradation than PEG.

This observation struck us as somewhat contradictory to the above-mentioned report on stability of oligo(L-proline) over PEG and inspired us to investigate the effects of ROS/RNS on other pseudo-polypeptides and compare the degradation behaviour with PEG. Thus, we compare the degradation of PEtOx, P(N-EtGly) and PEG with degrees of polymerization (DP) of approx. 50 and 120 (corresponding to 2–11 kg/mol) under conditions that have been described in the literature as being physiologically relevant.

2. Experimental

2.1. Materials

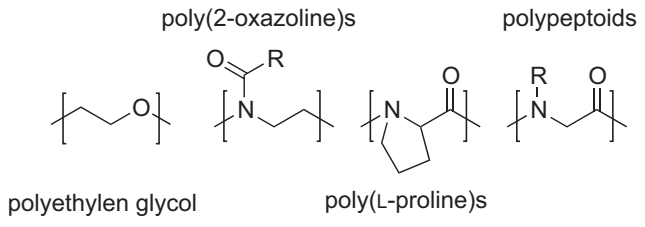
All chemicals and solvents were purchased from Sigma–Aldrich, Acros or Roth and used as received unless otherwise stated. Benzonitrile (PhCN) was dried by refluxing over P_2O_5, benzylamine over BaO, and MeOTf as well as 2-ethyl-2-oxazoline (EtOx) over CaH_2 under dry argon atmosphere and subsequent distillation prior to use. Acetonitrile (ACN) and chloroform were dried by storage over 3 Å and 4 Å molecular sieve, respectively. Poly(ethylene glycol) (M_n = 2000 g/mol, M_n^{GPC} = 22 kg/mol, $Đ$ = 1.07, Acros # 192280100 and M_n = 6000 g/mol, M_n^{GPC} = 47 kg/mol, $Đ$ = 1.06, Roth # 0154.1) did not contain radical inhibitors according to the providers and was used as received. Poly(N-ethylglycine) (POI-44: M_n^{GPC} = 31 kg/mol, $Đ$ = 1.05; POI-116: M_n^{GPC} = 57 kg/mol, $Đ$ = 1.06) and poly(2-ethyl-2-oxazoline) (POx-51: M_n^{GPC} = 15 kg/mol, $Đ$ = 1.09; POx-108: M_n^{GPC} = 21 kg/mol, $Đ$ = 1.08) were prepared as described previously [36,52].

2.2. Analytical methods

Gel Permeation Chromatography (GPC): GPC was carried out on a Polymer Standard Service (PSS, Mainz, Germany) system (MDS RI detector) using a 50 mm PFG precolumn and three 300 mm PFG columns (pore size 7 μm) for measurements in HFIP (containing 5 mmol/L ammonium trifluoroacetate). Columns were kept at 40 °C and the flow rate was set to 1 mL/min. Prior to each measurement, samples were filtered through 0.2 μm PTFE syringe filters (Roth, Karlsruhe, Germany). Calibration was performed using poly(methyl methacrylate) standards (PSS, Mainz, Germany) with molar masses from 800 g/mol to 1600 kg/mol. Data were processed using WinGPC software.

2.3. Degradation studies

Unless otherwise stated, degradation experiments were performed in phosphate buffered saline (PBS), which was prepared by dissolving sodium chloride (8.00 g, 137 mmol), potassium chloride (0.20 g, 2.68 mmol), disodium hydrogen phosphate (1.42 g, 10.0 mmol) and potassium dihydrogen phosphate (0.27 g, 1.98 mmol) in 1 L millipore water, yielding isotonic PBS with a pH value of 7.4 ± 0.01. Due to the poor solubility of PEtGly, PBS was diluted with millipore water to 25% of the initial concentration for degradation experiments with POI. Polymers were dissolved in PBS (1 g/L) and incubated over night at 37 °C to ensure full dissolution which was confirmed visually. Subsequently, $CuSO_4$ and different amounts of H_2O_2 were added to final concentrations of 50 μM $CuSO_4$ and 0.5, 5 and 50 mM H_2O_2, respectively. Samples were shaken at 37 °C and addition of H_2O_2 was repeated every 24 h in order to replenish degraded H_2O_2. At defined times, aliquots of 5 mL were withdrawn, immediately frozen in liquid nitrogen and subsequently freeze-dried for subsequent GPC analysis. It should be noted that this procedure may lead to the situation that some degradation products, which are potentially insoluble in HFIP are not analysed. However, in our experience this is not expected to a considerable extent as HFIP proofed an excellent solvent for a large variation of polymers.



Scheme 1. Structure of polymers studied or discussed. Polyethylene glycol is well known to be oxidatively labile while poly(2-oxazoline)s (POx) where discussed to be more stable as compared to the polyether. In contrast, oligo(L-proline), structurally similar to POx has been reported to be more labile as compared PEG. Polypeptoids, another structural analogue to POx and polypeptides, have not been studied in this context previously.

3. Results and discussions

As a polyether, PEG is prone to oxidative degradation [22,53–55]. However, most other organic substances, including proteins and other synthetic polymers also undergo oxidative degradation [1,2,56–58]. In the case of surface-bound polymer brushes, it has been demonstrated that PEG-based polymer brushes are less stable as compared to polymer brushes based on PMeOx [25]. Notably, the brushes comprised only very short oligomers of PEG and PMeOx, respectively (DP < 10). Here, we investigated the degradation of PEG, PEtOx (subsequently termed POx) and P(N-EtGly) (subsequently termed POI) with degrees of polymerization of approx. 50 and 120 which corresponds to molar masses of 2–11 kg/mol. As source for ROS we chose the system H_2O_2/Cu(II) as this was employed in one report which inspired this study [26]. Other researchers used alternatives such as H_2O_2/Co(II) or plain H_2O_2, the latter being used in the ISO 10993-13 standard [59]. In our opinion, none of these systems can be expected to be able to model the complex situation of ROS *in vivo* exactly (Fig. 1) but will certainly produce situations which may be encountered by soluble biomaterials in certain tissues, cells or cellular compartments. Aqueous copper solutions are able to catalyse degradation of H_2O_2 to more reactive ROS, such as hydroperoxide radical and hydroxyl radical (Scheme 2). However, these species only represent a fraction of the ROS encountered *in vivo*.

In the literature, different concentrations of H_2O_2 can be found to study oxidative degradation *in vitro* that is supposed to mimic conditions *in vivo*. While the ISO 10993-13 norm requires 3 wt% (approx. 1 M) H_2O_2 [60], Cosgriff-Hernandez and co-workers employed 20% H_2O_2 with 0.1 M $CoCl_2$. Sun and co-workers employed 5 mM H_2O_2 with 50 μM $CuSO_4$. We decided to employ the conditions of Sun et al. and in addition performed degradation studies at 50 and 0.5 mM H_2O_2 in phosphate buffered saline, with a polymer concentration of 1 g/L. After different times aliquots were retrieved and frozen at −20 °C or directly lyophilized. The appearance of the freeze-dried residue changes significantly over time from a typical fluffy polymer appearance to a typical salt-like appearance (Fig. S1).

3.1. Degradation analysis by GPC

The residues where subsequently dissolved in hexafluoroisopropanol (HFIP), insoluble components (e.g. buffer salts) removed by filtration, and the solutions analysed by gel permeation chromatography. At the highest ROS concentrations (50 mM, 0.2%(w/w)), rapid deterioration of all biomaterials is evidenced by a

$$Cu^{2+} + H_2O_2 \rightarrow Cu^{+} + \cdot OOH + H^{+}$$
$$H_2O_2 + Cu^{+} \rightarrow \cdot OH + OH^{-} + Cu^{2+}$$

Scheme 2. The initially added Cu(II)-ions are reduced to Cu(I) along with formation of hydroxyperoxide radicals. The Cu(I) ions subsequently reduce H_2O_2 to highly reactive hydroxyl radicals.

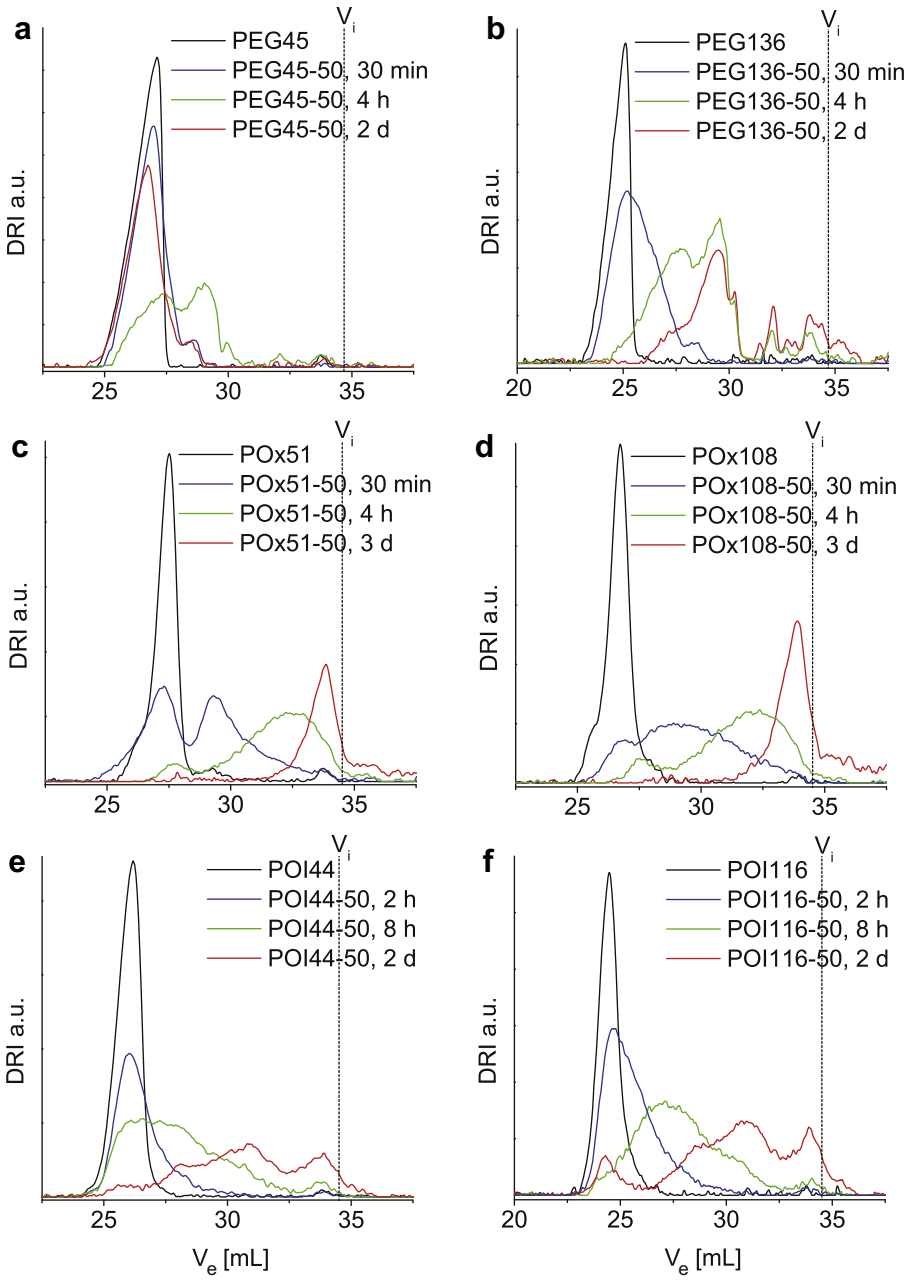


Fig. 3. Development of polymer elugrams (solvent HFIP) of polyethylene glycol (PEG), poly(2-ethyl-2-oxazoline) (POx) and poly(N-ethylglycine) (POI) with degrees of polymerization DP ≈ 50 (left panel, a,c,e) and DP ≈ 120 (right panel b,d,f) upon incubation with 50 mM of H_2O_2 and 50 μM of Cu(II)SO_4 at 37 °C for different periods of time. Please note, H_2O_2 was replenished once a day. V_i represents the column volume of the system.

rapid broadening of the elution peaks and its shift to higher elution volumes even after 30 min (Fig. 3). Even at lower concentration of 5 mM (Fig. 4) and 0.5 mM H_2O_2 (Fig. 5) clearly all three types of polymers are degraded, albeit slower. Interestingly, the elugrams of the different types of polymers seem to follow distinct patterns (Fig. 6). At 40–80% loss of initial M_w, the PEG and POx samples exhibit signature bimodal distributions while elugrams of POI samples only exhibit a signal broadening to higher elution volumes.

Plotting % residual M_w against time allows comparison of the degradation of the polymers at different [H_2O_2] (Fig. 7) and the different polymers at the same [H_2O_2] (Fig. 8). As expected and evident from GPC elugrams, rate of polymer deterioration increases with increasing [H_2O_2]. Moreover, the relative degradation clearly is higher for higher DP in all cases. This observation immediately rules out the possibility that the degradation exclusively occurs via chain-end scission, in which case smaller polymers must be expected to degrade more rapidly. At the highest ROS concentration (50 mM H_2O_2) polymers decayed very rapidly. At this concentration, the M_w is reduced by more than 50% within a few hours. At 5 mM H_2O_2, polymers are degraded to 50% of initial M_w ($t_{50\%}$) within 1–3 days for polymers with DP ≈ 120 and 2–6 days for polymers with DP ≈ 50. At the lowest concentration tested, $t_{50\%}$ ranged from 10 days (POx-51 and POI-44) to about 50 days (PEG-45) (Fig. 7, Table S1). These results were rather unexpected to us. To date, several reports state that POx is more stable as compared to PEG [25,50,51]. However, in the reports by Textor and co-workers, the

authors investigated surfaces coated with brush-polymers comprising poly(L-lysine)-graft-oligoethylene glycol and poly(L-lysine)-graft-oligo(2-methyl-2-oxazoline), respectively. The remaining polymer thickness upon incubation with 10 mM H_2O_2 (no transition metal catalysis) was investigated by ellipsometry. Therefore, a direct comparison to the present study is not possible. To see whether different species of ROS have different impact on polymer degradation, we also investigated the degradation of polymers in 50 mM H_2O_2 without Cu and in 50 μM Cu(II) without H_2O_2 (Fig. 9). Interestingly, no difference was observed between the samples in either case. While in 50 μM Cu(II) the M_w remained virtually unchanged over 20 days, all polymers degraded to about 25% of the initial M_w within 11 days in the presence of 50 mM H_2O_2. Thus, the oxidative degradation of the different families of polymers appears to be strongly dependent on the ROS employed. This is particularly interesting considering that POx was reported to be stable in aqueous solution under ambient conditions (i.e. presence of oxygen) while PEG supposedly forms hydroperoxides under such conditions [51]. As one reviewer has pointed out, it should be noted all polymers employed in this study may interact with Cu(II). However, in the absence of H_2O_2, the presence of Cu(II) clearly has no influence on the molar mass over prolonged periods of time (20 days). A copper catalysed hydrolysis is therefore not considered relevant in the observed time frame.

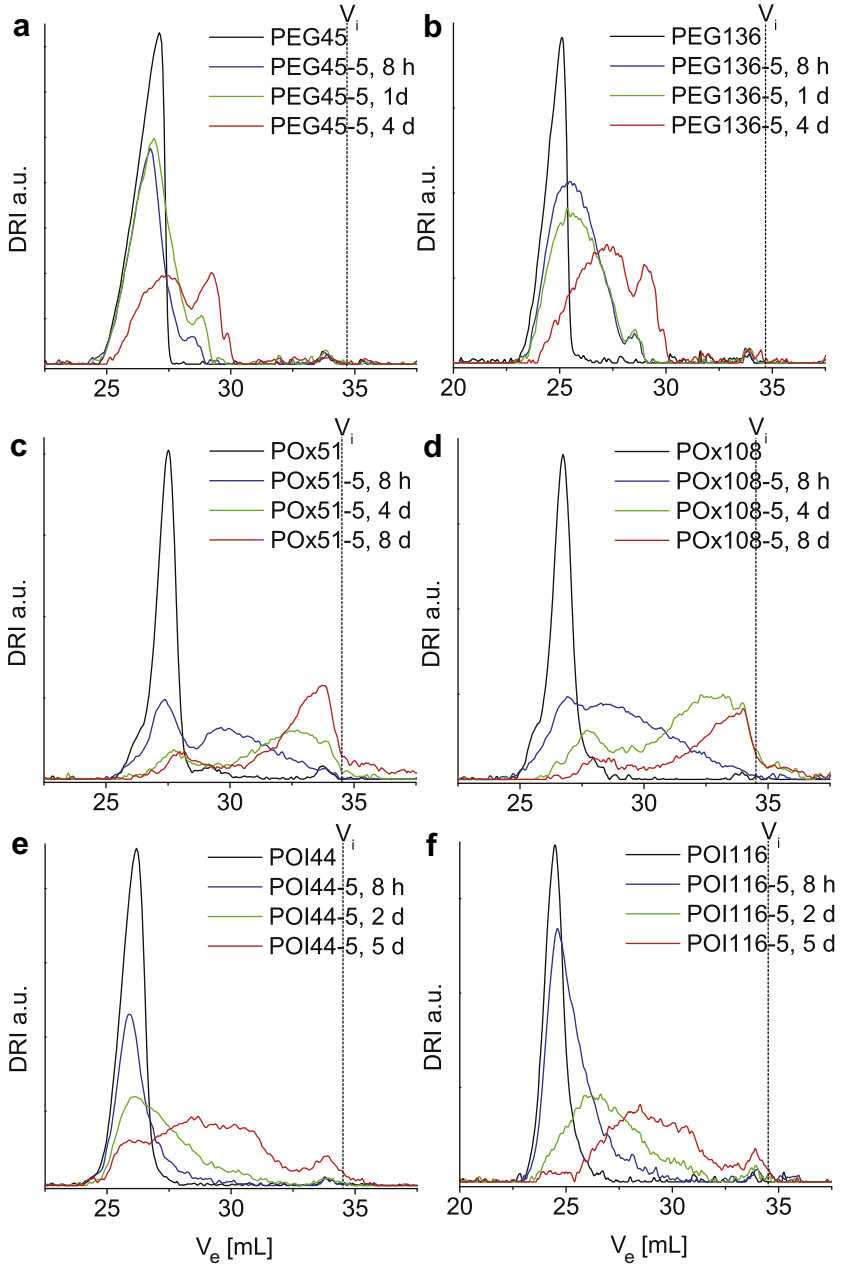


Fig. 4. Development of polymer elugrams (solvent HFIP) of polyethylene glycol (PEG), poly(2-ethyl-2-oxazoline) (POx) and poly(N-ethylglycine) (POI) with degrees of polymerization DP ≈ 50 (left panel, a,c,e) and DP ≈ 120 (right panel b,d,f) upon incubation with 5 mM of H_2O_2 and 50 μM of Cu(II)SO_4 at 37 °C for different periods of time. Please note, H_2O_2 was replenished once a day. V_i represents the column volume of the system.

3.2. Degradation mechanisms

The initial step in the degradation of all polymers will be abstraction of a hydrogen atom followed by addition of another radical or oxygen (Schemes 3 and 4). In the case of POx and POI, the location of initial H-abstraction will define whether the polymer backbone will be degraded (Scheme 3) or whether the side chains will be cleaved off (Scheme 4). In the case of PEG, formate esters and formaldehyde are potential products which may be further oxidized to carboxylates, which where identified by Friman and co-workers as metabolites of PEG after hepatic excretion [61]. In the case of polypeptoids, intermediate hydroperoxides may react via different pathways. Amides, formylamides and other reactive species are potential (intermediate) products. However, in contrast to polypeptide degradation, formation of isocyanates cannot occur, as this requires a secondary amine [28]. POx has two methylene groups in the backbone, which are chemically identical. Via pathways analogue to the oxidative degradation of proteins, the polymer chains can be degraded. In particular, POI is expected to degrade analogue to poly(L-proline) [57].

Considering the fact that in the case of PEG, H-abstraction and therefore bond scission can only occur in the polymer chain while in the case of POI and POx side chains can also be cleaved, it is rather surprising that the latter polymers are degraded faster compared to PEG. In fact, our results contradict the few reports available by others. However, we found that the degradation profile is strongly dependent on the DP and molar mass, respectively

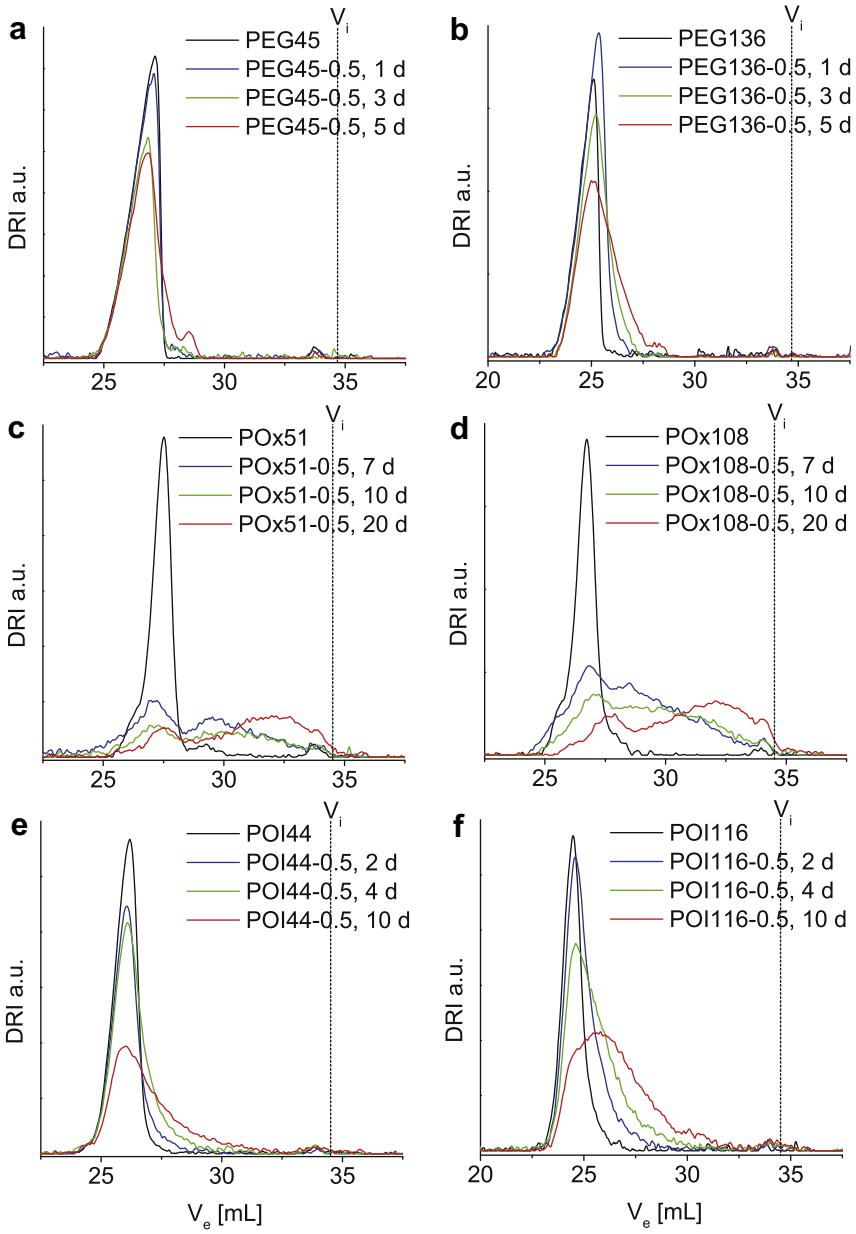


Fig. 5. Development of polymer elugrams (solvent HFIP) of polyethylene glycol (PEG), poly(2-ethyl-2-oxazoline) (POx) and poly(N-ethylglycine) (POI) with a degrees of polymerization DP ≈ 50 (left panel, a,c,e) and DP ≈ 120 (right panel b,d,f) upon incubation with 0.5 mM of H_2O_2 and 50 μM of Cu(II)SO_4 at 37 °C for different periods of time. Please note, H_2O_2 was replenished once a day. V_i represents the column volume of the system.

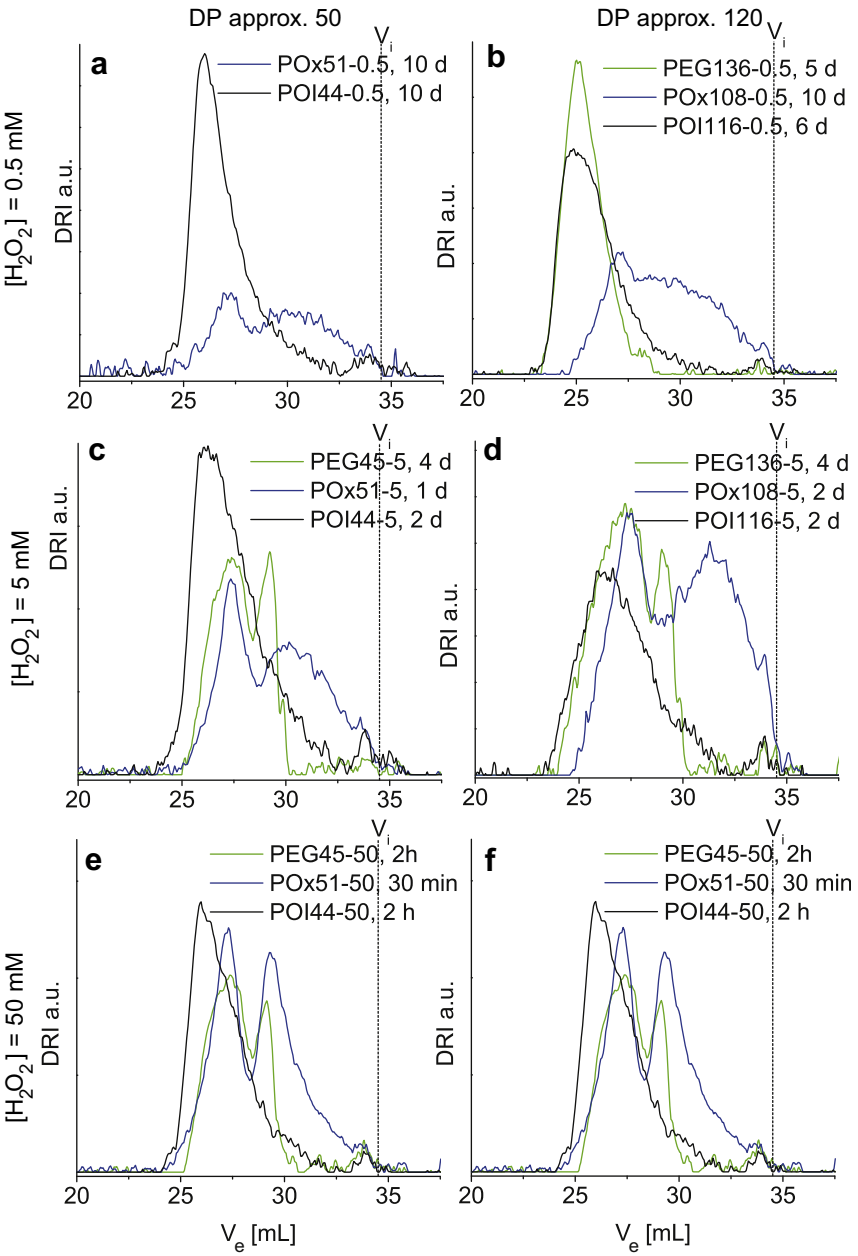


Fig. 6. Comparison of polymer elugrams (solvent HFIP) of polyethylene glycol (PEG), poly(2-ethyl-2-oxazoline) (POx) and poly(N-ethylglycine) (POI) with degrees of polymerization DP ≈ 50 (left panel, a,c,e) and DP ≈ 120 (right panel b,d,f) upon incubation with 0.5 mM (a,b) 5 mM (c,d) and 50 mM (e,f) of H_2O_2 and 50 μM of Cu(II)SO_4 at 37 °C for different periods of time to obtained a reduction of initial M_w of approx. 40–80%. V_i represents the column volume of the system.

(Fig. 10, Fig. S2). When relating to the DP, it becomes apparent that at 0.5 and 5 mM H_2O_2 the decline of half-live with increasing DP for PEG is much more pronounced as compared to POx and POI, which exhibit a very similar decline. However, the molar masses of the repeat units of the three polymers differ considerably (PEG: 44 g/mol, POI: 85 g/mol, POx: 99 g/mol). As a result, the plot of $t_{50\%}$ vs. molar mass gives a different picture. Here, the data points seem to fall together on one master curve, meaning that the degradation profile of PEG, POx and POI does not differ with respect to the polymer molar mass.

At 50 mM H_2O_2, this is not observed (Fig. S2) and POI appears to be more stable then PEG. Whether this is true or an artefact stemming from inaccuracies due to extremely fast degradation remains to be elucidated in further studies. However, considering that all experiments were carried out in triplicate, we assume that this is not an artefact.

3.3. Relation to materials with tailored oxidative responsive behaviour

Tirelli, Hubbell and co-workers have pioneered work with polypropylene sulfide (PPS), materials that are design to respond to oxidative stimulus [15–17,20,62–64]. Such materials hold great promise, for example in the context of ROS scavenging [64] and triggered drug release upon oxidative stimulus [18,26,65]. In this context, many studies employed PEG or pluronics as the hydrophilic component of the biomaterial. For example, Tirelli incubated PPS nanoparticles with a pluronic corona with 10% and 5% H_2O_2 (w/

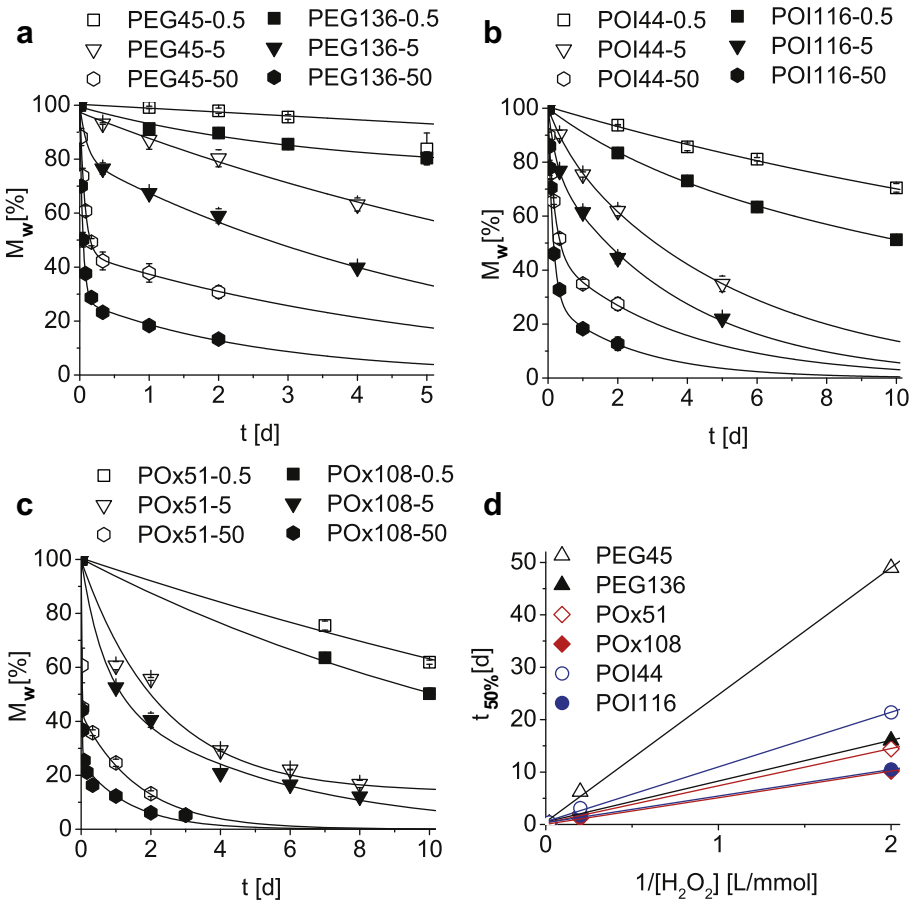


Fig. 7. Development of molar masses (a–c) (M_w relative to original M_w) of a) polyethylene glycol (PEG), b) poly(2-ethyl-2-oxazoline) (POx) and c) poly(N-ethylglycine) (POI) upon incubation with 0.5 mM, 5 mM and 50 mM of H_2O_2 and 50 μM of Cu(II)SO_4 at 37 °C as obtained from GPC elugrams. Please note, H_2O_2 was replenished once a day. d) Comparison of determined $t_{50\%}$ (with respect to molar mass M_w) of PEG, POx and POI plotted against 1/[H_2O_2]. Data (a–c) presented as means ± standard error means (SEM) ($n = 3$). Data were fitted with double exponential functions since single exponential functions did not yield fits of sufficient quality. Fits are only guide for the eyes.

w) for prolonged periods of time (>10 days) [16]. These concentrations are much higher than the highest concentrations we studied in the present contribution. All polymers studied were found to be significantly degraded after 11 days incubated in only 50 mM H_2O_2 (approx. 0.2% (w/w)).

Sung and co-workers studied the degradation of PEG-oligo(L-proline)–PEG polymers under similar conditions employed in the present work [26]. Within six days, the polymer was reported to converge on the molar mass of the used PEG (550 g/mol). It is difficult to relate this study to our present results, as two different polymers are combined in block copolymers and the degrees of polymerizations are small in comparison. However, the relatively high stability of PEG is corroborated by our studies but it may be doubted that PEG is completely stable under the investigated conditions.

In summary, PEG is used in a plethora of various biomaterials, which are discussed to be oxidation sensitive. To the best of our knowledge, PEG is more often than not considered to be stable, without stringent experimental proof. Our results suggest that a closer look at this issue is warranted.

3.4. Relation to in vitro and in vivo oxidative conditions

Oxidative degradation is an integral part of natural metabolism as well as host-defence mechanisms. However, ROS are also known to feed-back into cell signalling cascades and the influence on this process has been previously demonstrated. For example, Sung et al. demonstrated *in vitro* that insoluble polymeric biomaterials comprising PEG induced exogenous and intracellular ROS production, which was shown to trigger pro-apoptotic pathways [54]. However, the effect of PEG in biomaterials scaffolds is complex as was also demonstrated by the same group. Using biomaterial surfaces with different PEG content, it was demonstrated that low levels of PEG can induce apoptosis in attached cells. In contrast, higher PEG content protected cells from this fate [66]. In the view of the present work it would be interesting how materials with POx and POI would behave in such a context.

In addition to “simple” PEGylation of surfaces, proteins and drugs, PEG is used in uncountable studies in the field of polymer therapeutics and nanomedicine for the preparation of amphiphilic block copolymers, block ionomers or hydrogels [67–74]. For example, water-soluble block copolymers of PEG and polypropylene glycol (known as pluronics or poloxamers) have been studied as biomaterials for many years [75]. In some of this work, Kabanov and co-workers have found profound effects of pluronics on the respiratory process in mitochondria. These effects are typically closely related to the hydrophilic/lipophilic balance of the polymers [76–80]. However, in the light of the presented results, one can hypothesize that interaction with ROS, which is created in

significant amounts during respiration, may have an influence, too. In addition to the respiratory process, ROS are produced *in vivo* in many other processes. For example, NOX or DUOX produces ROS in response to growth factors, cytokines and calcium in a wide range of tissue [21].

For biomaterials, though, we hypothesize that the production of ROS by phagocytes will be much more relevant, as these are responsible for foreign body removal [81]. During phagocytosis, particles are engulfed by the phagocytic cell membrane, forming a vesicle inside the phagocyte, known as phagosome. Within the phagosome, particles and microorganisms are destroyed and digested by ROS generated by the phagocyte NOX2 enzyme. This so called respiratory burst is characterized by the production of superoxide and subsequent dismutation forming hydrogen peroxide [9,82]. Catalysed in enzymatic and non-enzymatic reactions, formation of more reactive ROS like hypochlorous acid or hydroxyl radicals [83] is feasible, following the ROS cascade shown in Fig. 1.

Winterbourn et al. estimated ROS concentrations in neutrophil phagosomes using a kinetic model describing the reactions of superoxide [84]. Although $\bullet O^{-2}$ is generated up to concentrations of 4 M, steady-state concentrations are considerably lower due to suggested reactions with myeloperoxidase (MPO) [85], yielding HOCl [86]. However, $\bullet O^{-2}$ is also converted to H_2O_2. As a result, micromolar steady-state levels of 2 μM H_2O_2 and 25 μM $\bullet O^{-2}$ are expected. Assuming a phagosomal MPO concentration around 1 mM, HOCl is

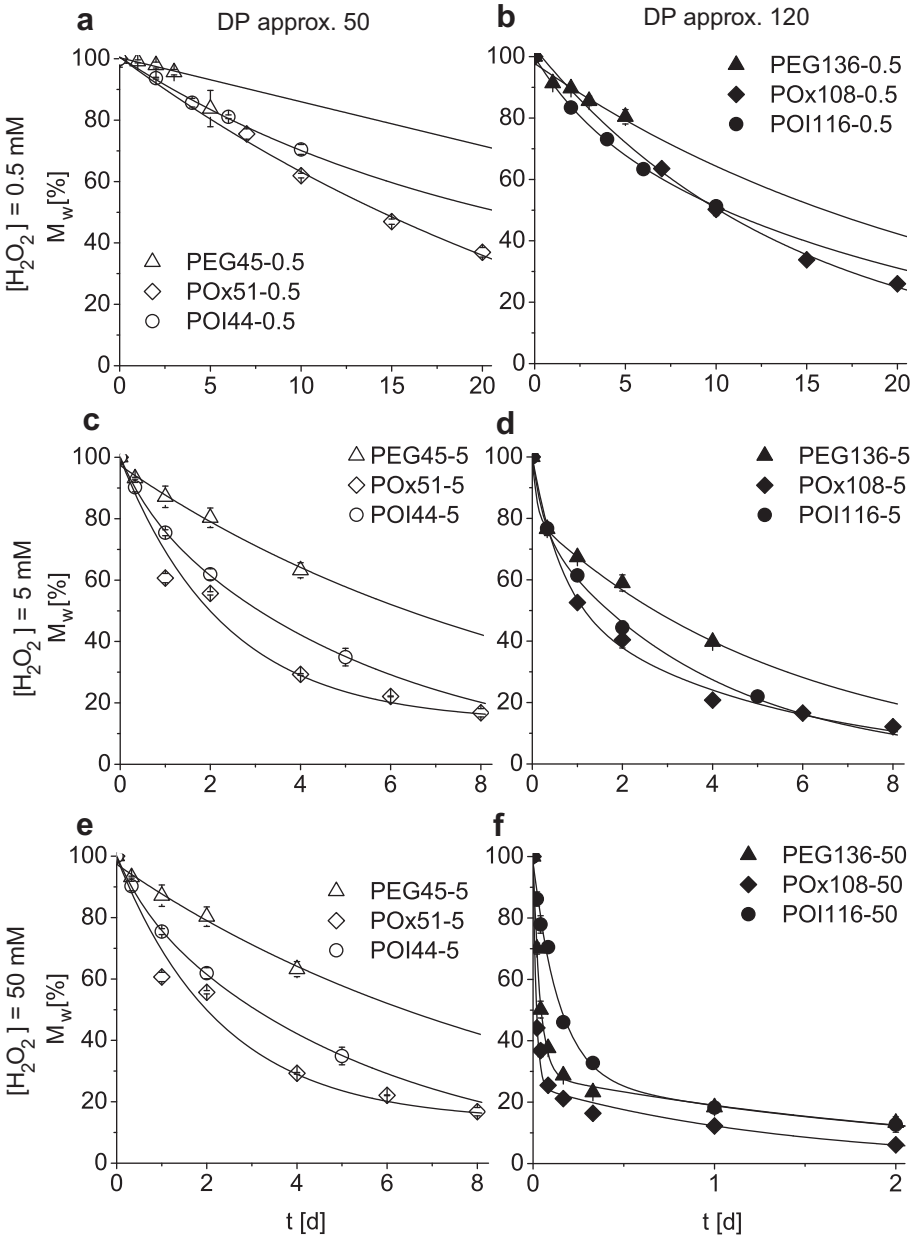


Fig. 8. Development of molar masses (a–c) (M_w relative to original M_w) of polyethylene glycol (PEG), poly(2-ethyl-2-oxazoline) (POx) and poly(N-ethylglycine) (POI) with a degrees of polymerization DP ≈ 50 (left panel, a,c,e) and DP ≈ 120 (right panel b,d,f) upon incubation with 0.5 mM (a,b), 5 mM (c,d) and 50 mM of H_2O_2 (e,f) and 50 μM of Cu(II)SO_4 at 37 °C for different periods of time. Please note, H_2O_2 was replenished once a day. Data presented as means ± SEM ($n = 3$).

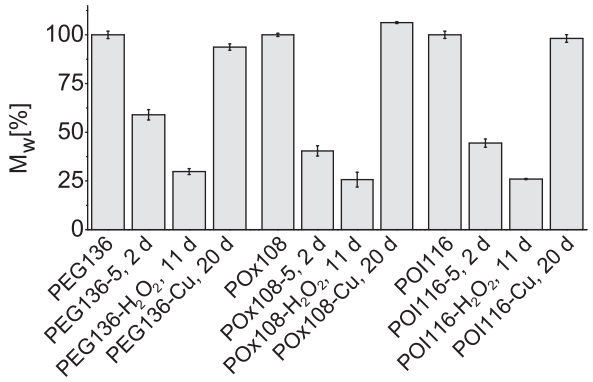
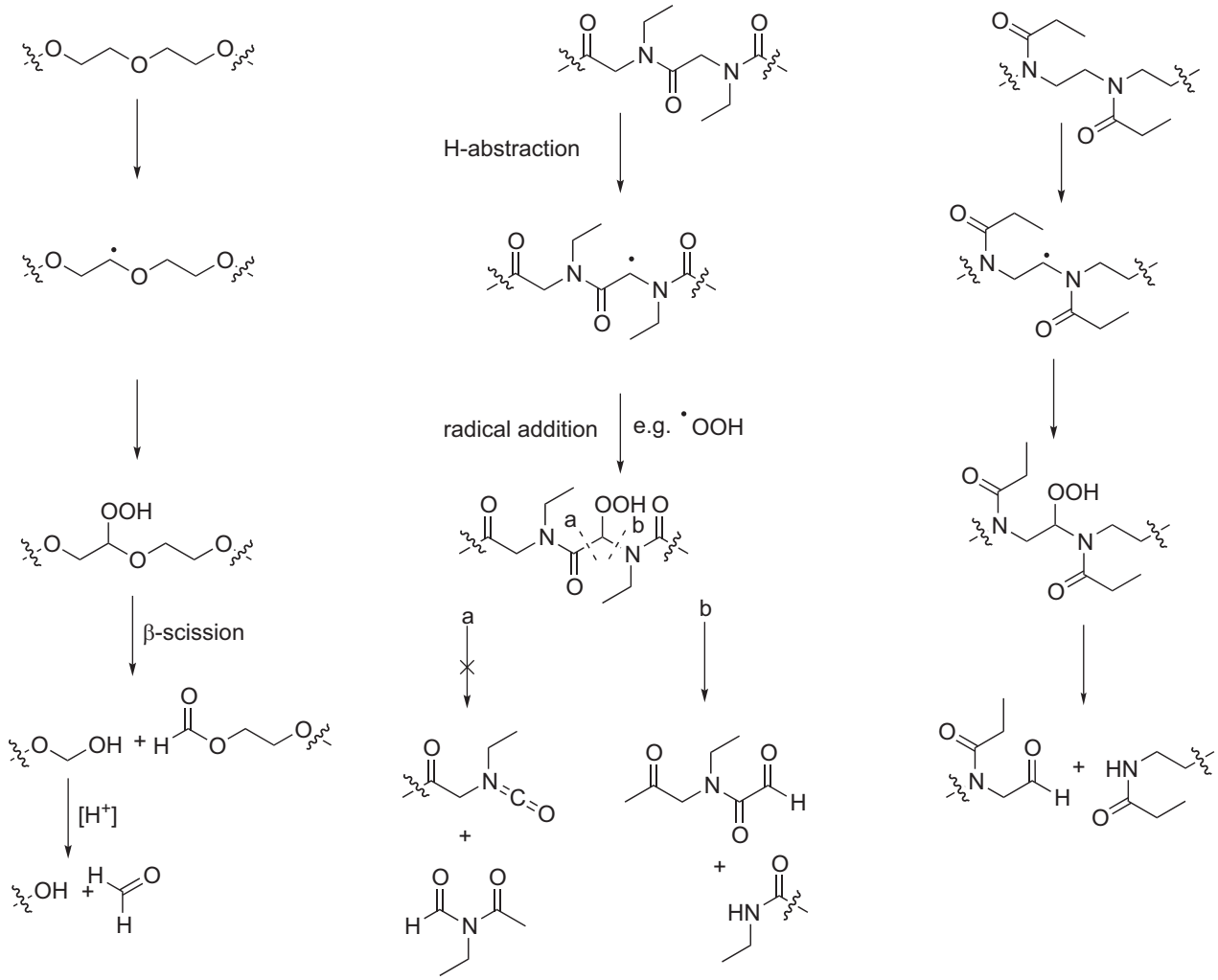


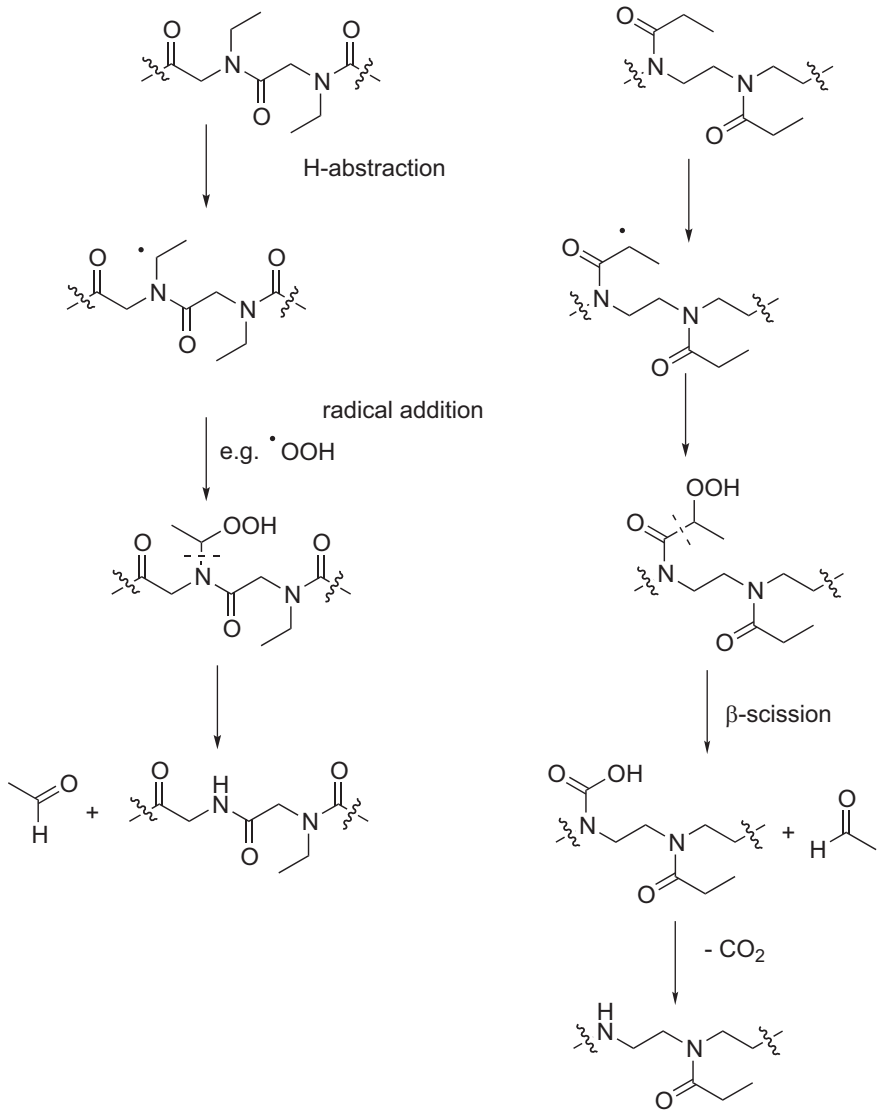
Fig. 9. Comparison of residual molar mass (M_w) of PEG, POx and POI dissolved in buffer, buffer with 5 mM H_2O_2 and 50 μM $CuSO_4$, buffer with 50 mM H_2O_2, and buffer with 50 μM $CuSO_4$. While neither buffer alone nor 50 μM $CuSO_4$ give appreciable polymer degradation, incubation with buffer containing 50 mM H_2O_2 degrades all polymers to approx. 25% of initial M_w. Data presented as means ± SEM ($n = 3$).

generated in rates of up to 134 mM/min, but likely the major part reacts with neutrophil proteins [87]. HOCl is a strong oxidant which is able to attack any oxidizable group [81]. Indeed, it is expected to be 50 times more effective in microbial killing than H_2O_2 [21]. The MPO/H_2O_2/Cl system is also capable of forming highly damaging hydroxyl radicals (·OH) as well as singlet oxygen ($^1O_2$), but both are reported to be minor species in the phagosome [8,11].

Macrophages differ from neutrophils regarding the predominant ROS produced. Within macrophages, nitric oxide synthase (NOS), more precisely iNOS, catalyses the formation of ·NO, a reactive nitrogen species (RNS) causing moderate toxicity [13,88]. In presence of superoxide, highly reactive peroxynitrite and hydroxyl radicals may be formed [89]. Prior to differentiation to macrophages, monocytes circulate with an estimated half life of 3 days [90]. Once migrated into tissue, differentiation to macrophages or dendritic cells follows [91]. Specific macrophages are located in almost all tissues, usually coupled with a distinct purpose. Representing approximately 60% of leukocytes, neutrophils may be primarily responsible for the degradation of polymers. After a short circulation half life of 6–8 h, neutrophils migrate into specific tissues, e.g. spleen, liver and bone marrow, where they are viable for only a few days [12]. Neutrophils are known to offer a



Scheme 3. Potential mechanisms for oxidative degradation of the polymer backbone of PEG, POx and POI. Please note, the depicted route represents only one out of many possibilities. A detailed description is outside the scope of this work.



Scheme 4. Potential mechanisms for oxidative degradation of the polymer side chain of POx and POI. Please note, the depicted route represents only one out of many possibilities. A detailed description is outside the scope of this work.

slightly higher ROS production rate than macrophages [92,93], which goes along with a higher antimicrobial capacity [94]. Both correspond with a loss of MPO during the maturation of monocytes to macrophages [11].

Considering the distribution and accumulation of polymer based drug delivery vehicles *in vivo*, phagocytes must be expected to play a role in their metabolism and degradation. Upon opsonization, particles may be absorbed and digested by neutrophils within the blood stream. Although PEG, POx and POI are all known to reduce opsonization in certain conditions, complete avoidance of the immune system is typically not realistic. It his hypothesized that once migrated into tissue, macrophages and dendritic cells could be responsible for the phagocytosis of polymer based vehicles that went off-target. More often than not, accumulation sites of polymers correspond well with the tissue distribution of macrophages. In the light of these considerations, the *in vivo* degradation of polymers by ROS generated in phagocytes should be considered in more detail in the future. The present study can only be seen as a starting point for much more detailed studies, which are clearly necessary.

4. Conclusion

Surprisingly little information can be found in the literature on the effect of oxidative degradation of hydrophilic biomaterials. Here, we presented a detailed study on the loss of molar mass of polyethylene glycol, poly(2-oxazoline)s and polypeptoids in aqueous buffer in the presence of different concentrations of ROS. We found that PEG is more stable than either at hydrogen peroxide concentrations below 50 mM when Cu(II)-catalyst is present. In contrast, in the absence of metal catalyst, no difference in the degradation was observed. Our results shed new light on the use of polyethylene glycol and the search for potential alternative biomaterials. A plethora of different ROS and RNS are present in the body in different locations, at different times and in different concentrations. In recent years, several researches have found evidence that polymers formerly thought biologically inactive may have intricate effects on cell membranes, signalling pathways and the respiratory chain. Our results may be a starting point of a much deeper understanding of the interplay of biological ROS and RNS with soluble biomaterials.

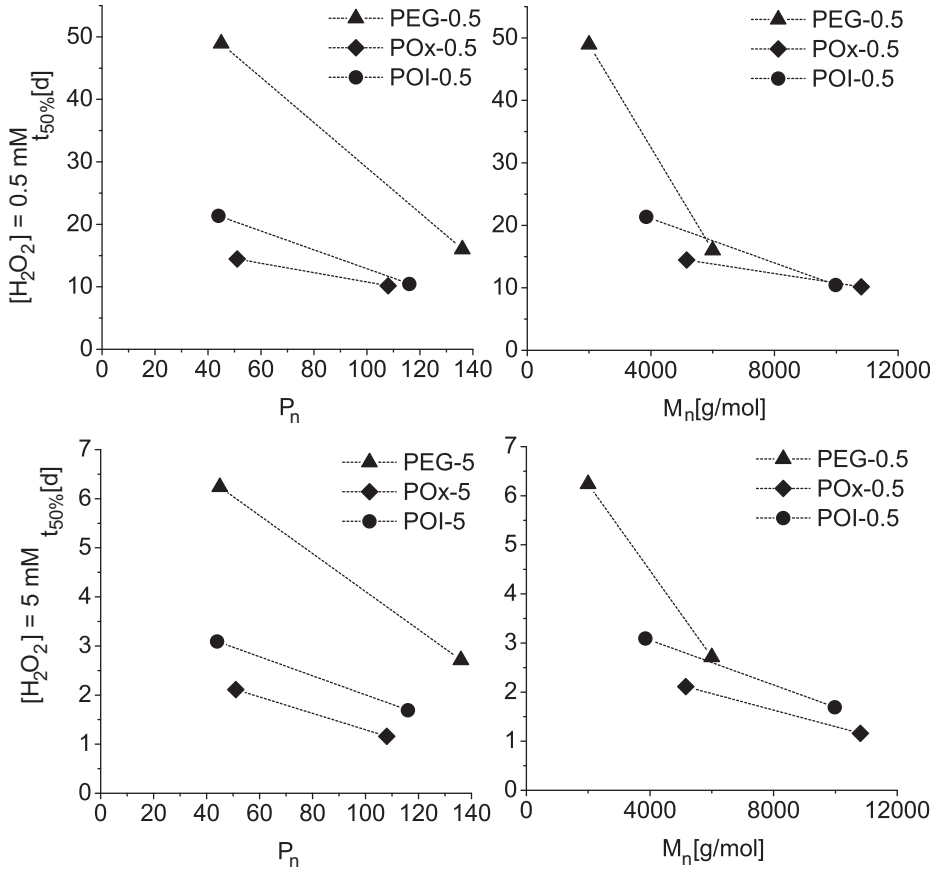


Fig. 10. Dependence of $t_{50\%}$ plotted against degree of polymerization P_n and against molar mass, respectively. Lines represent only a guide for the eye.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.02.029.

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