JOURNAL OF Applied Polymer SCIENCE

Understanding and tailoring the degradation of PVA-tyramine hydrogels

Khoon S. Lim, Justine J. Roberts, Marie-Helene Alves, Laura A. Poole-Warren, Penny J. Martens
Graduate School of Biomedical Engineering, University of New South Wales, Sydney, Australia
Correspondence to: P. J. Martens (E-mail: p.martens@unsw.edu.au)

ABSTRACT: We have previously reported on a hydrogel system fabricated from poly(vinyl alcohol) (PVA) functionalized with tyramine groups (PVA-Tyr) that has the ability to co-polymerize with proteins in their native state. These gels were also shown to be hydrolytically degradable through the ester groups present in the functional groups. In this article, the hydrolytic degradation of the PVA-Tyr gels is shown to be strongly dependant on pH, where at pH < 7.4 the lack of ionization of the tyramine groups resulted in slower hydrolysis. The gels' degradation was also highly influenced by temperature, where heat ($>25°C$) was required to facilitate the hydrolysis of the ester bonds. Moreover, the degradation rates were successfully tailored between 19 to 27 days by varying the hydrogels' initial macromer concentration. It was highlighted that the cross-linking density was dependant on the sodium persulphate to tyramine ratio, as well as the viscosity of the macromer solution. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42142.

KEYWORDS: biomaterials; degradation; photopolymerization

Received 15 January 2015; accepted 23 February 2015
DOI: 10.1002/app.42142

INTRODUCTION

Hydrogels are highly hydrated polymeric networks that have been extensively researched for various biomedical applications. In particular, synthetic hydrogels can be designed to be nondegradable or degradable depending on the targeted application. The majority of hydrolytically degradable hydrogels are engineered to contain ester linkages located either in the cross-link or the backbone of the hydrogel. The hydrolysis of these ester bonds has been widely studied in the literature where the esters undergo different hydrolysis mechanisms at different conditions (i.e., neutral, acid and base). The rate of ester hydrolysis is both pH and temperature dependent. The hydrolysis rate has been shown to generally increase with the acidity or alkalinity of the environment.[1] Also, it has been reported that heat can accelerate the hydrolysis rate by providing more energy to the reactants to overcome the activation energy required to initiate the hydrolysis reaction.[2–4] Interestingly, although the esters undergo different hydrolysis mechanisms at different conditions, similar end products, a carboxylic acid and an alcohol, are obtained.[4]

The hydrolysis kinetics of ester bonds also vary depending on the chemical structure of the ester,[5,6] as well as other factors such as polymer crystallinity and wettability.[7] It was reported that ester bonds associated with poly(lactic acid) (PLA) degrade much more rapidly than ester linkages of polycaprolactone (PCL).[8,9] Moreover, changes made to the polymer structure and size may also affect the degradation mechanisms. For example, bulk PLA has been shown to degrade in both acidic and basic environments, whereas PLA brushes only degrade in basic conditions.[10,11] Conversely, other studies of cross-linked polymers containing esters in the cross-link have demonstrated that their gels were nondegradable on the timeframes studied. Hennink *et al.* have reported that dextran hydrogels with high cross-linking density were resistive to hydrolysis despite having an ester group in the cross-links.[12] Hydrogels formed from poly(vinyl alcohol) (PVA) conjugated with an ester containing methacrylate moiety were also shown to be hydrolytically stable over a period of several months.[13,14]

One major advantage of hydrolytically degradable synthetic hydrogels is that the degradation rate of these gels can be tailored according to the targeted biomedical application. Previous studies have shown that hydrogels of tuned degradation profile can be engineered by varying the number of degradable linkages, macromer concentration and degree of cross-linking.[15] Martens *et al.* successfully tailored the degradation of PVA gels grafted with ester acrylate groups from 1 to 12 to 35 days by varying the macromer concentration from 10 to 15 to 20 wt % respectively.[15] Moreover, increasing the cross-linking density of the PVA ester acrylate gels from 0.3 to 0.64 mol/L by fabricating the gels using macromers of

Additional Supporting Information may be found in the online version of this article.

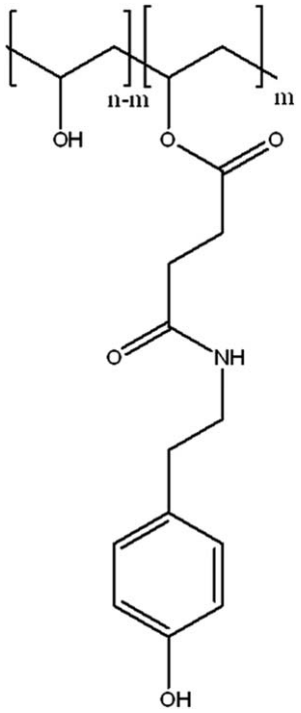

Figure 1. Schematic of PVA-Tyr; $n = 364$, $m = 7$.

different molecular weights also increased the degradation time from 12 to 45 days.[15] The ability to tune the degradation rate of these hydrogels is a major advantage in terms of designing tissue engineering matrices for various biomedical applications, such as drug delivery.[16] For example, tailoring the hydrogel degradation also controls the drug release rate, which then permits stable clearance of the drug from the body without exerting too much stress on the kidney.[17]

We have previously reported on a system that could covalently incorporate proteins into synthetic hydrogel networks without the need of prior chemical modification of the biological polymer. The synthetic base of the gel is composed of PVA functionalized with tyramine (Tyr) groups (PVA-Tyr) that can form covalent cross-links with tyrosine moieties of native proteins through a visible light photopolymerization technique.[18] The previous results have shown that these PVA-Tyr gels are hydrolytically degradable through the ester linkages located in the cross-links.[18] Although the PVA-Tyr gels have great potential as tissue engineering matrices, the degradation profile and characteristics of these gels remains unknown. Therefore, the aim of this article is to evaluate the degradation behavior of PVA-Tyr hydrogels in various incubation conditions (pH and temperature), as well as tailoring the degradation rate by changing the hydrogel macromer content.

MATERIALS AND METHODS

Materials

PVA (13–23 kDa, 98% hydrolyzed), succinic anhydride (SA), triethylamine (TEA), 1,3-Dicyclohexylcarbodiimide, N-hydroxysuccinimide, Tyr, sodium persulphate (SPS), 1,1-carbonyldiimidazole, tris(2,2-bipyridyl)dichlororuthenium(II) hexahydrate (Ru(II)bpy_3^{2+}), deuterium oxide (D_2O), molecular sieves (4 Å), Dulbecco's phosphate buffered saline (DPBS), dialysis tubing (10 kDa molecular weight cutoff), Eagle's minimum essential media (EMEM), trypsin, fetal bovine serum, and penicillin streptomycin were purchased from Sigma-Aldrich and used as received. Dimethyl sulfoxide was bought from Ajax Chemicals and was dried over 4 Å molecular sieves. Hydrogel disc moulds were made from silicone sheets (Silastic®Sheeting, reinforced medical grade silicone rubber, Dow Corning).

Macromer Preparation

Synthesis of PVA-Tyr. PVA-Tyr (Figure 1) was synthesized according to a two-step reaction previously published.[18] Briefly, carboxyl groups were conjugated onto the PVA backbone using SA and TEA. Tyr moieites were then conjugated to the carboxylated PVA (PVA-COOH) using a conventional carbodiimide–amine coupling reaction. The by-product (dicyclohexylurea) formed during the reaction was removed using vacuum filtration. The filtered PVA-Tyr solution was further purified by dialysis against water, and then freeze-dried. The PVA-Tyr used in this article was characterized to be 2% tyraminated (7 Tyr per PVA chain) using ^{1}H NMR.

Fabrication of PVA-Tyr Hydrogels. Dried PVA-Tyr was dissolved in DPBS at 80°C. Upon complete dissolution, the polymer solution was cooled to room temperature (RT) and the initiators, Ru (2 m*M*) and SPS (20 m*M*) were added to the solution. The macromer solution was then placed into silicon moulds on a glass slide and covered with a cover slip. The samples were then irradiated under 30 mW/cm^2 of visible light (400–450 nm) (Blue wave 200, Dymax) in a closed system.

Swelling and Mass Loss Analysis

Directly after polymerization, all samples were weighed for the initial wet mass (m_{initial}) and three samples were immediately lyophilized to obtain their dry weights ($m_{\text{dry,t0}}$). The actual macromer fraction was calculated based on the equation below:

$$\text{Actual macromer fraction} = \frac{m_{\text{dry},\ t=0}}{m_{\text{initial},\ t=0}} \quad (1)$$

These samples were then submerged in a sink of DPBS or saline (9 g of sodium chloride in 1 L of water) solution and incubated. Samples were removed from the incubator after 1 day, blotted dry and weighed (m_{swollen}). The swollen samples were then freeze-dried and weighed again (m_{dry}). The mass swelling ratio (q) and mass loss were calculated as follows:

$$q = \frac{m_{\text{swollen}}}{m_{\text{dry}}} \quad (2)$$

$$m_{\text{initial, dry}} = m_{\text{initial}} \times \text{actual macromer fraction} \quad (3)$$

$$\text{Mass loss} = \frac{m_{\text{initial,dry}} - m_{\text{dry}}}{m_{\text{initial,dry}}} \times 100 \quad (4)$$

The sol fraction is given by the mass loss at 1 day, where previous studies conducted in the lab have shown that noncross-linked polymers will dissolve out from the hydrogel network (incubated in 37°C and DPBS) within this time frame.[14,19] Images of the gels were taken using the Leica M80 stereo microscope at 0.75× magnification. The samples were monitored

daily until complete degradation was observed. The time required for complete degradation was measured.

The effective macromer percentage at day 1 was calculated by the equation below:

$$\text{Effective macromer percentage} = \frac{m_{\text{dry},\ t=1}}{m_{\text{swollen},\ t=1}} \times 100 \quad (5)$$

The cross-linking density (ρ_x) was calculated using the equations below:[20,21]

$$Q = 1 + \frac{\rho_{\text{polymer}}}{\rho_{\text{solvent}}}(q-1) \quad (6)$$

$$\frac{1}{\overline{M_c}} = \frac{2}{\overline{M_n}} - \frac{\left(\frac{\bar{v}}{V_1}\right)\left[\ln(1-v_{2,s}) + v_{2,s} + \chi v_{2,s}^2\right]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{\frac{1}{3}} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \quad (7)$$

$$v_{2,s} = \frac{1}{Q} \quad (8)$$

$$v_{2,r} = \frac{1}{Q_{t=0}} \quad (9)$$

$$\rho_x = \frac{1}{\bar{v}\overline{M_c}} \quad (10)$$

where $\overline{M_c}$ is the number average molecular weight between cross-links, $\overline{M_n}$ the number average molecular weight in the absence of any cross-linking (16,000 g/mol for PVA), $\bar{v}$ the specific volume of the polymer (0.788 cm^3/g for PVA),[22,23] V_1 the molar volume of the solvent (18 mL/mol for PBS[24]), $v_{2,s}$ the equilibrium polymer volume fraction, $v_{2,r}$ the polymer volume fraction after cross-linking but before swelling, χ the polymer solvent interaction (0.494 for PVA in water),[22,23] M_r the molecular weight of the repeating unit (44 g/mol for PVA),[22] and C_n the characteristic ratio (8.9 for PVA).[22]

The assumptions associated with these equations are: tetrafunctional arrangement of the cross-links, Gaussian distribution of the cross-linked polymer chains, and the formation of an ideal network (no cyclization and chain interaction).[22,25]

Effect of Different Incubation Conditions on Degradation of PVA-Tyr Hydrogels

Effect of pH on Hydrolytic Degradation of PVA-Tyr Hydrogels. Fabricated 20 wt % PVA-Tyr hydrogels were immersed in saline solutions of various pH (2, 6, 7.4, 10, and 12), then incubated at 37°C. The mass loss and mass swelling ratios of the gels were calculated using eqs. (1–4). The pH was also measured at time points 1, 3, 7, 10, 14, and 21 days.

Effect of Temperature on Hydrolytic Degradation of PVA-Tyr Hydrogels. Fabricated 20 wt % PVA-Tyr hydrogels were immersed in DPBS (pH=7.4) and incubated at various temperatures (20°C, 30°C, 37°C, 50°C and 60°C). At the predetermined time points, the mass loss and mass swelling ratios of the samples were calculated using eqs (1–4).

Effect of Varying Macromer Concentration on Physical Properties of PVA-Tyr Hydrogels

Tailoring the Degradation of PVA-Tyr Hydrogels. PVA-Tyr hydrogels of 10, 15, and 20 wt % were fabricated as outlined in Fabrication of PVA-Tyr Hydrogels section. The sample names correspond to the nominal initial wt % of the gels (e.g., 10% PVA-Tyr). The gels were then immersed in DPBS (pH = 7.4) and incubated at 37°C. Equations (1–4) were used to calculate the mass loss and mass swelling ratios corresponding to the fabricated samples.

Measuring the Viscosity of PVA-Tyr Macromer Solutions. The viscosity of PVA-Tyr macromer solutions was obtained using a Kinexus Pro Rheometer (Malvern) with a cone and plate arrangement (Temperature = 21°C, Gap = 100 μm, Frequency = 1 Hz and Strain = 0.005%).

Compression Testing. The mechanical properties of the PVA-Tyr hydrogels were characterized using unconfined uniaxial compression testing at room temperature. Samples (5 mm diameter × 1 mm thick) were immersed in DPBS and incubated at 37°C. At predetermined time points (0, 1, 3, 7, and 10 days) the samples were removed from DPBS and compressed at a strain rate of 1 mm/min using an Instron 5543 mechanical tester. The slope of the linear regression of the stress–strain curve generated within 5–15% strain was used to calculate the compressive modulus (K).

Statistical Analysis

All samples for each study were prepared in triplicates, and each study was repeated three times. Minitab 15 statistical analysis software was used to perform two-way ANOVA on the results. The same software was also used to conduct general regression analysis on slopes of data sets collected for mass loss and swelling studies.

RESULTS AND DISCUSSION

Degradation of PVA-Tyr Hydrogels at Different Incubation Conditions

Effect of pH on Degradation Profile of PVA-Tyr Hydrogels. Previously published results have shown that PVA-Tyr hydrogels are hydrolytically degradable through the ester bonds present in the cross-links.[18] However, literature suggests that the hydrolysis rates and mechanisms of ester bonds vary at different conditions; therefore, the behavior of PVA-Tyr hydrogels at neutral, acidic, and basic conditions was examined in this study. It was observed that the PVA-Tyr gels did not degrade in acidic environments (pH = 2 and 6, Figure 2), and the mass loss remained constant in the range of ~20–30% throughout the study.

The initial loss likely corresponds to the noncross-linked macromers (sol fraction) diffusing out from the gel, and agrees with previous sol fraction values reported for these kind of gels (25.6 ± 4.79%).[18] At pH = 7.4, hydrogel degradation occurred with a linear degradation profile where the gels completely dissociated within 19 days. In a basic environment the degradation was accelerated, where the samples were completely degraded within 5 and 1 day at pH = 10 and 12, respectively (Figure 2). For carboxylic esters and phosphoester groups it has been shown that increasing acidity *or* alkalinity resulted in faster hydrolysis rates.[1,3] Conversely, the PVA-Tyr hydrogels in this study only degraded in pH ≥ 7.4. This observation suggests that although degradation occurred at the ester bonds present in the hydrogel network, other chemical groups around the ester

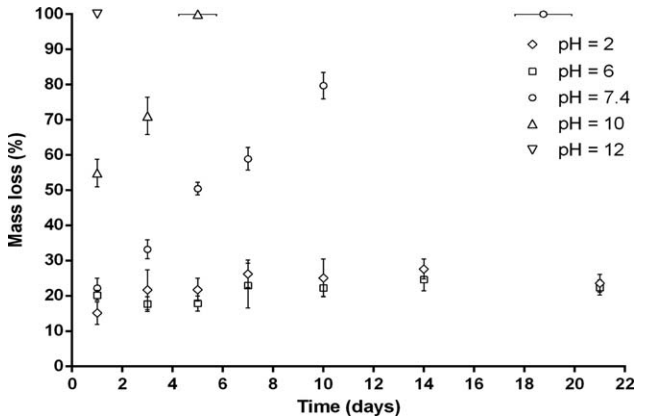

Figure 2. Mass loss profiles of PVA-Tyr gels at different pHs. Temperature was kept constant at 37°C. Vertical error bars represent standard deviation for mass loss (%) while horizontal error bars represent standard deviation of degradation period. No significant differences ($P > 0.05$) were observed between samples in pH 2 and 6 for all time points. Regression analysis showed that the slopes of samples in pH 7.4, 10, and 12 were statistically different ($P < 0.05$).

bonds, such as the aromatics/phenols of the Tyr moieties might have affected the hydrolysis. Ghandehari *et al.* also showed that hydrogels containing azoaromatic cross-links were stable in acidic environment but degradable in basic conditions.[26] It was speculated that these gels had low levels of ionization in an acidic environment which hindered the accessibility of the degradable bonds, thus no degradation occurred.[26] Similarly, hydrogels containing weak acid groups such as carboxylic acid were also reported to be pH sensitive with higher ionization levels in basic media compared to acidic media.[26–29] For example, hyaluronic acid hydrogels cross-linked through hydrazides were shown to be stable in acid but degradable in basic environments because of the different ionization levels of pendant carboxyl groups in the network.[28] The higher ionization level leads to greater water uptake into the gels, which subsequently cause faster degradation.[28] As the pendant phenol groups in PVA-Tyr are also weak acids, it was hypothesized in this study that the lack of ionization in acidic solutions was the cause of the reduced degradation observed at pH < 7.4. This statement agrees with previous work done by Riegelman *et al.* where the degree of ionization of the phenol groups of phenylephrine and Tyr was shown to increase with pH.[30]

Similarly, the mass swelling ratios (q) of PVA-Tyr hydrogels incubated in acidic saline remained constant over the time period of the study (Figure 3). This result agrees with the mass loss study where no degradation occurred at pH = 2 and 6. At pH = 7.4 and 10, it was shown that the q increases over time linearly as per the degradation profile. As the hydrogel is degrading, the cross-links are being cleaved leading to formation of larger mesh sizes that allow more water to be imbibed in the network.[31] Similarly, the q in basic conditions (pH = 10) are significantly higher when compared to physiological conditions (pH = 7.4) at respective time points (Figure 3). Once again it was speculated that more water was entrapped in the PVA-Tyr hydrogels in basic conditions because of the higher ionization level.

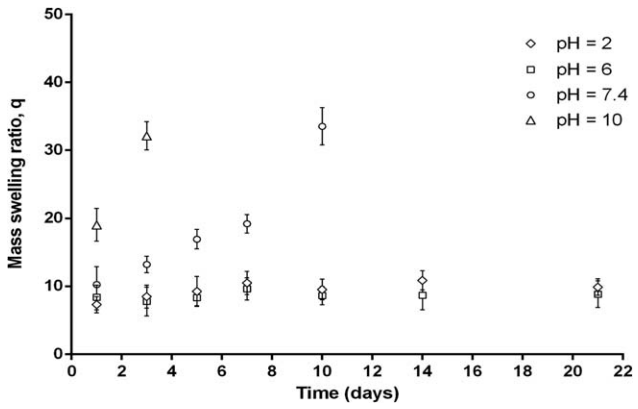

Figure 3. Mass swelling ratio, q of PVA-Tyr gels at different pH. The temperature was kept constant at 37°C. No significant differences ($P > 0.05$) were observed between samples in pH 2 and 6 for all time points. Regression analysis showed that the slopes of samples in pH 7.4, 10, and 12 were statistically different ($P < 0.05$).

Effect of Temperature on Degradation Profile of PVA-Tyr Hydrogels. It has been reported in the literature that heat/temperature affects the hydrolysis rate of ester bonds therefore the effect of temperature on the degradation rates of PVA-Tyr hydrogels was also examined. For these studies, the pH was kept constant at 7.4. No sign of degradation was observed when the PVA-Tyr gels were kept at 20°C, where mass loss values were consistent with the sol fraction percentage (~20–30%) for all the time points (Figure 4). When the temperature was increased to 30°C, a slow degradation was noted. Further increasing the temperature to 37°C, 50°C, and 60°C yielded linear degradation profiles where the gels were all completely degraded within 19, 3 and 1 day, respectively (Figure 4). These observations agree with the literature where ester hydrolysis at neutral pH can be accelerated with heat. Xu *et al.* showed that PLA hydrogel brushes degraded more rapidly at higher temperature.[10] Comisar *et al.* also reported that hydrolysis rate constants of esters increase with temperature.[32] At elevated temperature, more thermal energy is provided to the system to

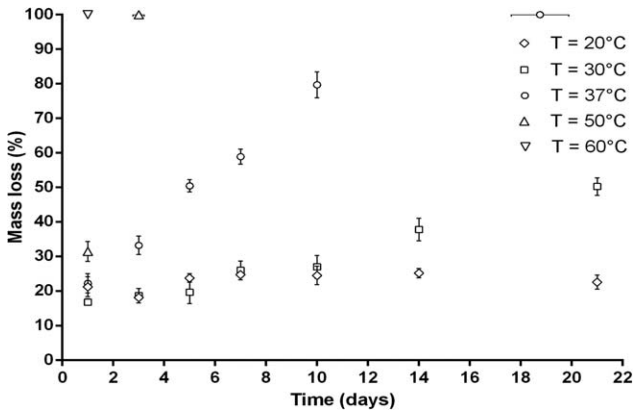

Figure 4. Mass loss profiles of PVA-Tyr gels at various temperatures. The pH was kept constant at 7.4. Vertical error bars represent standard deviation for mass loss (%) while horizontal error bars represent standard deviation of degradation period. The slopes of all degradation profiles were statistically different ($P < 0.05$).

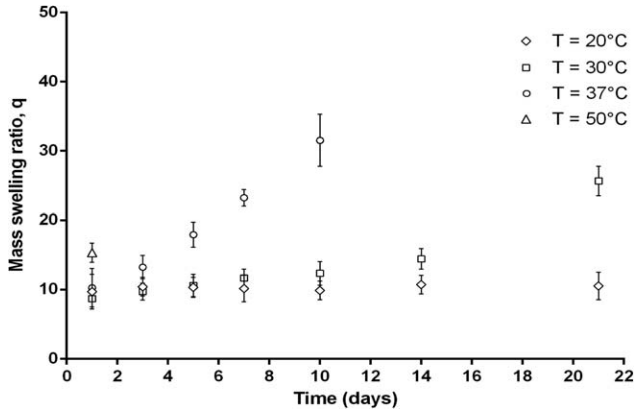

Figure 5. Mass swelling ratio, q of PVA-Tyr gels at different temperatures. The pH was kept constant at 7.4. Regression analysis of the swelling behavior showed that samples were statistically different at each temperature examined ($P < 0.05$).

facilitate the hydrolysis of ester bonds, hence the faster degradation rate achieved.[2]

Again the swelling values correlate with the mass loss data. The q of PVA-Tyr gels remained constant (~10) at 20°C, which was the temperature where no degradation was observed (Figure 5). As degradation only started to happen when heat was applied to the system, the increase in q over time was also only observed at elevated temperatures (30°C, 37°C, and 50°C).

Tailoring Degradation of PVA-Tyr Hydrogels by Varying Macromer Concentration

It has been shown in other systems that the degradation rates of hydrogels can be tailored by altering the macromer content.[15,33,34] In this study, the degradation profiles of 10%, 15%, and 20% PVA-Tyr hydrogels were examined. The pH (7.4) and temperature (37°C) were kept constant for all three macromer contents.

It was shown that 10% PVA-Tyr has the lowest sol fraction (15% sol fraction), followed by 15% PVA-Tyr (20% sol fraction) and 20% PVA-Tyr (22% sol fraction) (Table I). The sol fraction has generally been reported to decrease with increasing nominal macromer concentration.[35] However, the results in this study are in contrasts with those findings. The effective macromer percentage, which is defined as the ratio of the mass of cross-linked macromers in the hydrogel to the total mass of the hydrogel (mass of cross-linked macromers and mass of water in the hydrogel) after equilibrium swelling (1 day) was also calculated [eq. (5)]. Interestingly, the samples with the lowest nominal macromer concentration (10% PVA-Tyr) ended up with the highest effective macromer percentage (~12 wt %), which was significantly different to both 15% and 20% PVA-Tyr gels (~10 wt % effective) after equilibrium swelling (Table I). This observation indicates that after equilibrium swelling and sol fraction extraction, the total amount of macromer in the 10% PVA-Tyr gels was higher than both the 15% and 20% PVA-Tyr gels. Furthermore, the swelling (q) of the fabricated samples was also shown to increase with higher nominal macromer concentration (Table I), which agrees with the trend observed for the sol fraction and effective macromer percentage values. This result was further supported by the macroscopic images of PVA-Tyr hydrogels taken directly after polymerization (before swelling, $t = 0$) and after 1 day of swelling. It was clearly seen that 10%, 15%, and 20% PVA-Tyr gels had similar dimensions before swelling (Figure 6). After 1 day, 20% PVA-Tyr hydrogels were observed to swell the most, as compared to 15% and 10% PVA-Tyr gels (Figure 6).

This difference in swelling can be explained by the cross-linking density which is known to directly correlate to the swelling behavior of the gels [eqs. (5–8)].[22,36] The 10% PVA-Tyr hydrogels had the lowest mass swelling ratio and the lowest sol fraction, which equates to the highest cross-linking density when compared to 15% PVA-Tyr and 20% PVA-Tyr gels (Table I). In a tighter network (i.e., higher cross-linking density) the mobility of the polymeric chains are more restricted, and thus less water can be absorbed in the network.[34,37,38] Zustiak *et al.* showed that the swelling of PEG-based hydrogels decreased with increasing cross-linking density.[5] Burdick *et al.* also reported that increasing cross-linking density of methacrylated hyaluronan hydrogels resulted in smaller mesh size and lower swelling.[37] Moreover, statistical analysis revealed that the cross-linking densities were significantly different for all three compositions.

The increased sol fraction and swelling that was observed for gels with higher nominal macromer percentage in this study is because of the relative initiator concentration present in the gels.[39] In this system, Ru^{2+} photo-oxidizes to Ru^{3+} by donating an electron to SPS during the polymerization process. The excited Ru^{3+} then reverts back into Ru^{2+} by abstracting electron from the Tyr groups attached to the PVA, generating tyrosyl radicals that are responsible for the cross-link. Therefore, the cross-linking cascade terminates when the entire electron accepting SPS has been consumed. As the concentration of SPS

Table I. Physical Properties of 10%, 15% and 20% PVA-Tyr Hydrogels

Nominal macromer concentration (wt %)	Sol fraction (%)	Effective macromer percentage (wt %)[a]	Mass swelling ratio, q	Cross-linking density, ρ_x ($\times 10^4$ mol/L)[b]	Viscosity (mPa s)
10% PVA-Tyr	14.85 ± 2.65	12.19 ± 0.62	8.14 ± 0.56	2.95 ± 0.19	22 ± 1.2
15% PVA-Tyr	19.07 ± 4.24	10.65 ± 0.95	9.46 ± 0.84	2.39 ± 0.17	90 ± 5.0
20% PVA-Tyr	21.37 ± 3.36	9.97 ± 0.72	10.1 ± 0.77	2.11 ± 0.13	326 ± 30.2

[a] As determined from the mass loss and swelling at 24 hours (Eq. (5)).
[b] Calculated from the equations (6–10).

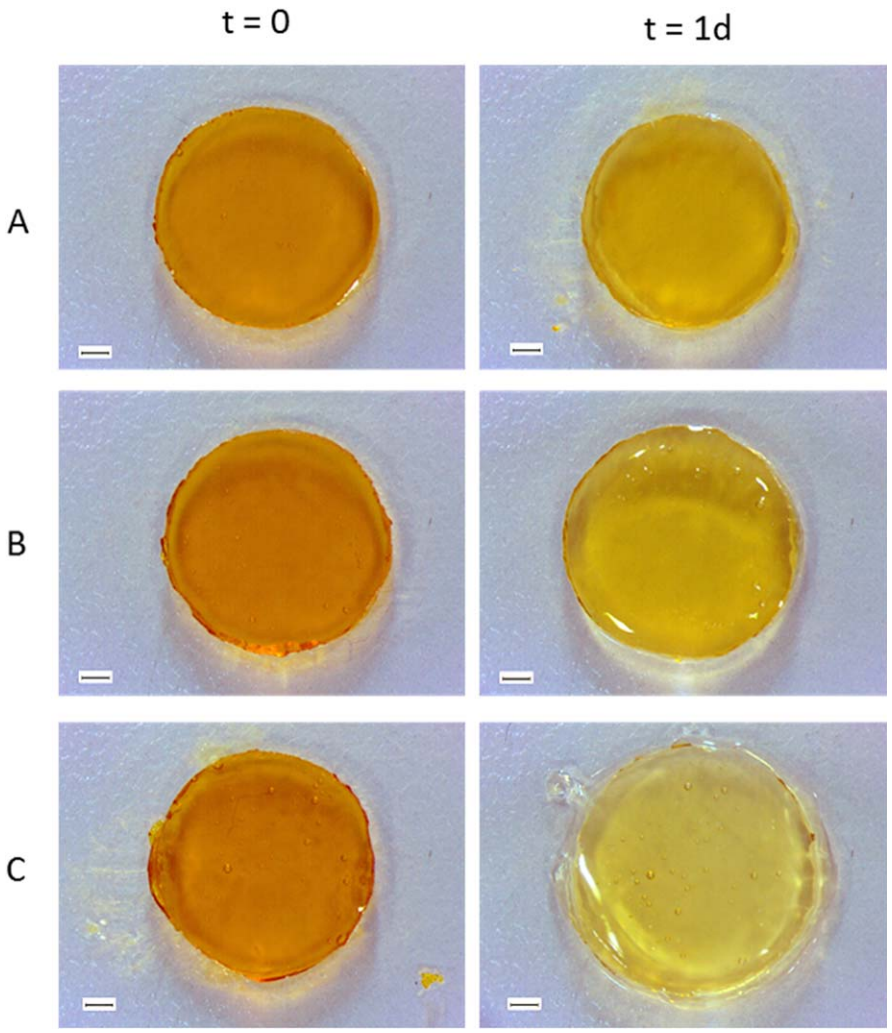

Figure 6. Macroscopic images of PVA-Tyr gels fabricated at $t = 0$ (before swelling) and 1d (after swelling); A = 10% PVA-Tyr; B = 15% PVA-Tyr; C = 20% PVA-Tyr; Scale bar = 1 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was kept constant at 20 m*M* for all three nominal hydrogel macromer concentrations in this study, the molar ratios of SPS to Tyr functional groups were actually highest in the 10% PVA-Tyr gels (0.46 : 1) followed by 15% (0.31 : 1) and 20% PVA-Tyr (0.23 : 1) hydrogels. This change in initiator: functional group ratio was hypothesized to be the main reason for the changes in sol fraction and degradation. In a separate study this was tested and it was shown that increasing the SPS: Tyr ratio did successfully decrease the sol fraction of 10% PVA-Tyr hydrogels until a saturation point (see Supporting Information Table S1 and Figures S1 and S2). However, this decrease was not observed in the 20% PVA-Tyr hydrogels. The difference observed between the 10% and 20% PVA-Tyr samples is hypothesized to be because of the viscosity of the initial macromer solutions. During fabrication, the PVA-Tyr macromer solutions' viscosity dramatically increased with macromer concentration (Table I). It was observed that the 20% PVA-Tyr macromer solution has a viscosity of 326 mPa s, which is approximately 15-fold higher than the 10% PVA-Tyr (~22 mPa s). This was not unexpected as it has previously been shown that PVA solutions with higher concentrations have higher viscosities.[25,40–42] This increase in viscosity reduced the cross-linking efficiency by affecting the mobility of macromer chains during photopolymerization. During the cross-linking process, the Tyr groups conjugated to the PVA chains are being converted to tyrosyl radicals to facilitate cross-links formation. Therefore, the increase in overall solution viscosity restricts the mobility of the PVA chains and their pendant tyrosyl radicals and thus limited the cross-linking reaction. In addition, an increase in viscosity is known to have an impact on the reaction rate constant.[43]

Mass loss studies revealed that 10%, 15%, and 20% PVA-Tyr hydrogels were completely degraded in ~ 27, 22, and 19 days, respectively (Figure 7). This phenomenon can again be explained by the differences in the effective macromer fraction and the cross-linking density of the fabricated gels. Theoretically, the number of cross-links formed is directly proportional to the cross-linking density.[38,44–46] Furthermore, the swelling capacity of the gel also decreases because of the tighter network formed, causing the hydrolysable ester bonds to be less accessible to water molecules.[33,34,47,48] Hence, 10% PVA-Tyr hydrogels which were determined to have the highest effective macromer concentration

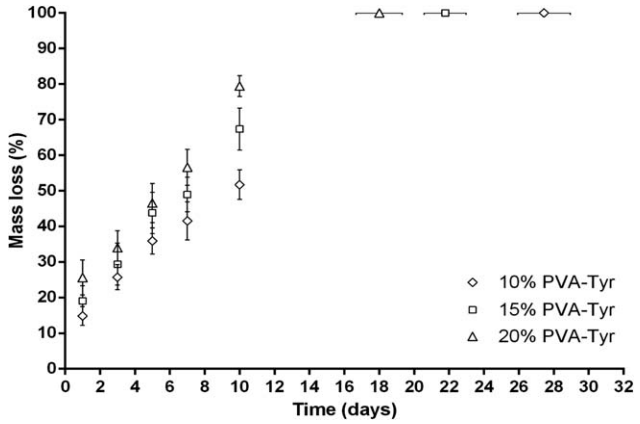

Figure 7. Mass loss profiles of 10%, 15%, and 20% PVA-Tyr gels at physiological conditions. Vertical error bars represent standard deviation for mass loss (%) while horizontal error bars represent standard deviation of degradation period. No statistical significance was observed at time points 1, 3, 5 and 7 days ($P > 0.05$). About 10%, 15%, and 20% PVA-Tyr gels were significantly different at 10 days ($P < 0.05$). The time to complete degradation was also statistically different for all three compositions ($P < 0.05$). The slopes of degradation profiles for 10%, 15%, and 20% PVA-Tyr gels were statistically different ($P < 0.05$).

(~12%) and to be the most cross-linked gel would be expected to degrade at a slower rate compared to 15% and 20% PVA-Tyr (both ~10% effective gels). A study conducted by Burdick *et al.* showed that increasing the cross-linking density of methacrylated hyaluronan hydrogels resulted in longer degradation period.[37] Similarly, Martens *et al.* showed that increasing the cross-linking density of PVA ester acrylate gels also resulted in slower degradation.[15] Furthermore, Lee *et al.* revealed that tyraminated hyaluronan hydrogels had a slower degradation rate when the cross-linking density was increased.[49]

The mass swelling ratios of the gels followed the same trend as the mass loss profiles (Figure 8). During degradation, the cross-links in the hydrogel are cleaved, leading to a larger mesh and

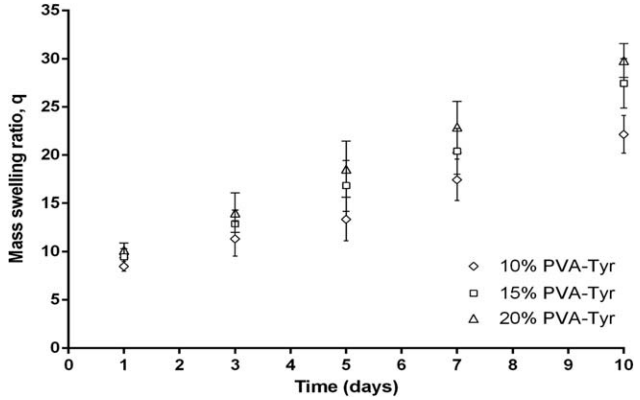

Figure 8. Mass swelling ratio, q of 10%, 15%, and 20% PVA-Tyr gels at physiological conditions. No statistical difference was observed for time points 1, 3, 5, and 7 days ($P > 0.05$).At 10 days, 10% PVA-Tyr was significantly different to 15% and 20% PVA-Tyr ($P < 0.05$). Regression analysis on the swelling profiles for 10%, 15%, and 20% PVA-Tyr gels showed statistically different slopes ($P < 0.05$).

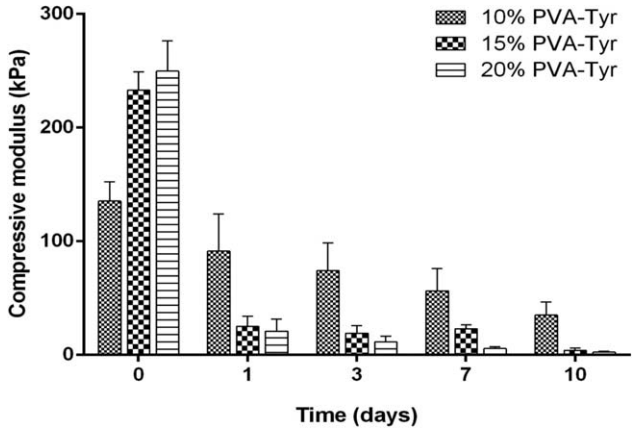

Figure 9. Compressive modulus of 10%, 15%, and 20% PVA-Tyr hydrogels at various time points. 10% PVA-Tyr was statistically different to 15% and 20% PVA-Tyr at 0, 1, and 3 days ($P < 0.05$). All three compositions were significantly different at 7 and 10 days ($P < 0.05$).

more water uptake (i.e., increase in mass swelling ratio). About 10% PVA-Tyr gels were found to exhibit a lower mass swelling ratio at all the time points studied, followed by 15% and 20% PVA-Tyr samples. This result is in accordance to previous sections where 10% PVA-Tyr gels had the slowest degradation rate. Another possible explanation for this observation is the different mol content of charged Tyr groups present in the hydrogels of various macromer concentrations, as increasing the number of ionic groups in hydrogels has been reported to increase their swelling capacities.[50,51] Previous work has shown that incorporating charged molecules, such as heparin and chondroitin sulphate, into the hydrogel network caused an increase in swelling.[19] Durmaz *et al.* also showed that increasing the ionic group concentration in polyacrylamide gels from 0 to 80 mol % resulted in a 27-fold increase in the volumetric swelling ratio.[51] This increment was explained by the overall increase of counter-ions in the gels, causing an additional osmotic pressure that swells the gel.[50] As 20% PVA-Tyr hydrogels have the highest concentration of Tyr groups in the network, the anionic repulsion between these moieties can also lead to a larger mesh and higher water content.[52,53]

Mechanical Properties of PVA-Tyr Hydrogels with Various Macromer Concentrations. The hydrogel's mechanical properties during degradation were also studied. At $t = 0$, the compressive modulus of the fabricated hydrogels increased in relation to the nominal macromer concentration (Figure 9). About 10%, 15%, and 20% PVA-Tyr gels had compressive modulus of ~135, 233, and ~250 kPa, respectively. This result was expected at this time point as all the macromer chains (cross-linked and noncross-linked) were still present in the gel. As the total amount of macromer in the 10% PVA-Tyr is the least, it was expected that the compressive modulus would also be the lowest. This result is in agreement with a study conducted by Martens *et al.* where the modulus was shown to increase with the amount of macromer in the hydrogel.[25] After immersing the gels into DPBS for 1 day to extract the sol fraction and allow equilibrium swelling, all the gels had a major decrease in the compressive modulus. This loss was because of the removal of

noncross-linked macromer chains (sol fraction) which leads to a decrease in the total amount of polymer chains in the final hydrogel network.[25,48,54] Moreover, at day 1 the 10% PVA-Tyr exhibited a compressive modulus of ~90 kPa which was much higher than 15% (~25 kPa) and 20% PVA-Tyr gels (~20 kPa). This observation was now expected, because of the higher effective macromer concentration and more tightly cross-linked network. Bryant *et al.* showed that increasing gel cross-linking density from 0.119 to 0.376 mol/L resulted in gels with 11-fold higher compressive modulus.[55] Moreover, all gels had a decrease in compressive modulus throughout the degradation period. It has been demonstrated in the literature that during degradation, hydrolysis of network chains cause a decrease in the degree of cross-linking, which further reduces the gels' mechanical strength.[37,56]

CONCLUSION

In conclusion, it has been demonstrated that the hydrolytic degradation of PVA-Tyr hydrogels is strongly related to the pH and temperature of the degradation medium. It was also highlighted that PVA-Tyr hydrogels with lower nominal macromer concentration resulted in gels with higher effective macromer fraction and cross-linking density. The cross-linking efficiency of this system is highly influenced by the initiator to functional groups ratio, as well as the viscosity of the macromer solution, which further influenced the degradation rates and mechanical strength of the fabricated PVA-Tyr hydrogels.

ACKNOWLEDGMENTS

This research was supported by an Australian Research Council Discovery Project Grant (DP0986447). J. J. R. was supported by a Whitaker International Scholarship Grant and University of New South Wales (UNSW) Early Career Research Grant.

REFERENCES

1. Bergmann, F.; Segal, R.; Shimoni, A.; Wurzel, M. *Biochem. J.* **1956**, *63*, 684.
2. Simanenko, Y. S.; Belousova, I. A.; Savelova, V. A.; Popov, A. F.; Vakhitova, L. N. *Theor. Exp. Chem.* **2004**, *40*, 17.
3. da Silva, P. L.; Guimarães, L.; Pliego, J. R. *J. Phys. Chem. B* **2013**, *117*, 6487.
4. Hilal, S. H.; Karickhoff, S. W.; Carreira, L. A.; Shrestha, B. P. *QSAR Comb. Sci.* **2003**, *22*, 917.
5. Zustiak, S. P.; Leach, J. B. *Biotechnol. Bioeng.* **2011**, *108*, 197.
6. Carvalho, J.; Moreira, S.; Maia, J.; Gama, F. M. *J. Biomed. Mater. Res. A* **2010**, *93*, 389.
7. Janorkar, A. V.; Metters, A. T.; Hirt, D. E. *Macromolecules* **2004**, *37*, 9151.
8. Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581.
9. Nicodemus, G.; Bryant, S. J. *Tissue Eng. B* **2008**, *14*, 149.
10. Xu, L.; Crawford, K.; Gorman, C. B. *Macromolecules* **2011**, *44*, 4777.
11. Jung, J. H.; Ree, M.; Kim, H. *Catal. Today* **2006**, *115*, 283.
12. Hennink, W. E.; De Jong, S. J.; Bos, G. W.; Veldhuis, T. F. J.; Van Nostrum, C. F. *Int. J. Pharm.* **2004**, *277*, 99.
13. Cavalieri, F.; Miano, F.; D'Antona, P.; Paradossi, G. *Biomacromolecules* **2004**, *5*, 2439.
14. Lim, K. S.; Kundu, J.; Reeves, A.; Poole-Warren, L. A.; Kundu, S. C.; Martens, P. J. *Macromol. Biosci.* **2012**, *12*, 322.
15. Martens, P.; Holland, T.; Anseth, K. S. *Polymer* **2002**, *43*, 6093.
16. Pradal, C.; Grøndahl, L.; Cooper-White, J. J. *Biomacromolecules* **2014**, *16*, 389.
17. Zustiak, S. P.; Leach, J. B. *Biomacromolecules* **2010**, *11*, 1348.
18. Lim, K. S.; Alves, M. H.; Poole-Warren, L. A.; Martens, P. J. *Biomaterials* **2013**, *34*, 7097.
19. Nilasaroya, A.; Poole-Warren, L. A.; Whitelock, J. M.; Jo Martens, P. *Biomaterials* **2008**, *29*, 4658.
20. Canal, T.; Peppas, N. A. *J. Biomed. Mater. Res.* **1989**, *23*, 1183.
21. Martens, P.; Anseth, K. S. *Polymer* **2000**, *41*, 7715.
22. Nafea, E. In: Graduate School of Biomedical Engineering; University of New South Wales: Australia, **2012**.
23. Peppas, N. A.; Merrill, E. W. *J. Polym. Sci. Polym. Chem. Ed.* **1976**, *14*, 441.
24. Martinez-Lopez, A. L.; Carvajal-Millan, E.; Lizardi-Mendoza, J.; Lopez-Franco, Y. L.; Rascon-Chu, A.; Salas-Munoz, E.; Barron, C.; Micard, V. *Molecules* **2011**, *16*, 8410.
25. Martens, P.; Blundo, J.; Nilasaroya, A.; Odell, R. A.; Cooper-White, J.; Poole-Warren, L. A. *Chem. Mater.* **2007**, *19*, 2641.
26. Ghandehari, H.; Kopečkové, P.; Kopeček, J. *Biomaterials* **1997**, *18*, 861.
27. Akala, E. O.; Kopečková, P.; Kopeček, J. *Biomaterials* **1998**, *19*, 1037.
28. Vercruysse, K. P.; Marecak, D. M.; Marecek, J. F.; Prestwich, G. D. *Bioconjugate Chem.* **1997**, *8*, 686.
29. Qiu, Y.; Park, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 321.
30. Riegelman, S.; Strait, L. A.; Fischer, E. Z. *J. Pharm. Sci.* **1962**, *51*, 129.
31. Meyvis, T. K. L.; De Smedt, S. C.; Demeester, J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 4717.
32. Comisar, C. M.; Hunter, S. E.; Walton, A.; Savage, P. E. *Ind. Eng. Chem. Res.* **2007**, *47*, 577.
33. Martens, P. J.; Bowman, C. N.; Anseth, K. S. *Polymer* **2004**, *45*, 3377.
34. Martens, P. J.; Bryant, S. J.; Anseth, K. S. *Biomacromolecules* **2003**, *4*, 283.
35. Moeinzadeh, S.; Khorasani, S. N.; Ma, J.; He, X.; Jabbari, E. *Polymer (Guildf)* **2011**, *52*, 3887.
36. Gehrke, S. H.; Fisher, J. P.; Palasis, M.; Lund, M. E. *Ann. N. Y. Acad. Sci.* **1997**, *831*, 179.
37. Burdick, J. A.; Chung, C.; Jia, X.; Randolph, M. A.; Langer, R. *Biomacromolecules* **2005**, *6*, 386.
38. Peppas, N. A.; Huang, Y.; Torres-Lugo, M.; Ward, J. H.; Zhang, J. *Annu. Rev. Biomed. Eng.* **2000**, *2*, 9.

39. Elvin, C. M.; Brownlee, A. G.; Huson, M. G.; Tebb, T. A.; Kim, M.; Lyons, R. E.; Vuocolo, T.; Liyou, N. E.; Hughes, T. C.; Ramshaw, J. A. M.; Werkmeister, J. A. *Biomaterials* **2009**, *30*, 2059.
40. Hassan, C.; Peppas, N. In Biopolymers PVA Hydrogels, Anionic Polymerisation Nanocomposites; Springer: Berlin Heidelberg, **2000**; p 37.
41. Modarress, H.; Nia, M. M.; Mostafa, R. *Iran. Polym. J.* **2005**, *14*, 181.
42. Kadajji, V. G.; Betageri, G. V. *Polymers* **2011**, *3*, 1972.
43. Olea, A. F.; Thomas, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 4494.
44. Asmussen, E.; Peutzfeldt, A. *Eur. J. Oral Sci.* **2001**, *109*, 282.
45. Peppas, N. A. Hydrogels in Medicine and Pharmacy, Peppas, N. (Ed.) CRC: BocaRaton, FL, **1987**.
46. Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. *Adv. Mater.* **2006**, *18*, 1345.
47. Bryant, S. J.; Bender, R. J.; Durand, K. L.; Anseth, K. S. *Biotechnol. Bioeng.* **2004**, *86*, 747.
48. Nuttelman, C. R.; Henry, S. M.; Anseth, K. S. *Biomaterials* **2002**, *23*, 3617.
49. Lee, F.; Chung, J. E.; Kurisawa, M. *J. Control. Release* **2009**, *134*, 186.
50. Flory, P. J. Principles of Polymer Chemistry; Cornell University Press: New York, **1953**.
51. Durmaz, S.; Okay, O. *Polymer* **2000**, *41*, 3693.
52. Sitte, H. H.; Huck, S.; Reither, H.; Boehm, S.; Singer, E. A.; Pifl, C. *J. Neurochem.* **1998**, *71*, 1289.
53. Lee, B.-D.; Lee, M.-J. *Bull. Korean Chem. Soc.* **2009**, *30*, 787.
54. Nuttelman, C. R.; Tripodi, M. C.; Anseth, K. S. *Matrix Biol.* **2005**, *24*, 208.
55. Bryant, S. J.; Anseth, K. S.; Lee, D. A.; Bader, D. L. *J. Orthop. Res.* **2004**, *22*, 1143.
56. Behravesh, E.; Timmer, M. D.; Lemoine, J. J.; Liebschner, M. A. K.; Mikos, A. G. *Biomacromolecules* **2002**, *3*, 1263.