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Biodegradable networks for soft tissue engineering by thiol-yne photo cross-linking of multifunctional polyesters

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Abstract

Novel biodegradable networks have been prepared by combination of post-polymerization modification of poly(ε-caprolactone) and thiol-yne photo cross-linking. In a first step, a novel multifunctional poly(ε-caprolactone) bearing propargyl groups (PCL-yne, 8 mol% substitution degree) was obtained in a rapid one-pot reaction and at a multigram scale. In a second step, PCL-yne and mixtures of PCL and PCL-yne were cross-linked via photoradical thiol-yne reaction in the presence of pentaerythritol tetrakis(3-mercaptopropionate) and various photoinitiators. The thermal properties and stabilities of the obtained networks have been evaluated before assessment of their mechanical properties. Finally, biocompatibility studies are reported with evaluation of the proliferation of L929 fibroblasts on the materials.

Keywords: thiol-yne chemistry; polyester; post-modification; photo cross-linking; degradable networks

1. Introduction

Degradable elastomeric and shape memory polymeric biomaterials used in FDA Class III medical devices only emerged in the late 1990s and are highly desirable for the preparation of tissue engineering scaffolds.[1-4] Among them, biomaterials based on aliphatic polyesters, poly(ε-caprolactone) (PCL), poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their block copolymers are of particular interest, which explains that to date they remain the most frequently utilized resorbable synthetic polymers in biomedical applications, especially tissue engineering.[5] As a consequence, numerous studies have dealt on the preparation of degradable cross-linked polyesters. In particular the well-known step-growth polymerization strategy, historically used to prepare biostable thermosets, has been applied to synthetize a family of polycondensates based on polyols and citric acid or sebacic acid.[6, 7] However, although being highly biocompatible, most of these materials have shown limited mechanical properties and fast degradations, which limits their application to engineering tissues with rapid healing rate.[2, 4, 8] A second strategy relying on the cross-linking of prepolymers functionalized by appropriate reactive groups has also been exploited for the preparation of cross-linked copolyesters. In most cases, advantage is taken from the hydroxyl and carboxylic chain ends to yield cross-linkable linear telechelic copolyesters with curable acrylate moieties.[9-14] Applied to PLA-PEG-PLA triblock copolymers this approach enabled us to prepare elastomeric materials exhibiting tunable mechanical and degradation properties.[11] Alkene groups have also been advantageously used in thiol-ene chemistry to cross-link materials as this type of reaction belongs to the click-chemistry tool-box.[15, 16] Various authors have therefore proposed thiol-ene thermal- or photo cross-linking of copolyesters for the preparation of gels, mainly based on PLA/PEG copolymers,[17-20] or elastomeric materials as exemplified by the cross-linking of telechelic PCLs,[21] globalide/CL copolyesters,[22] dendrimeric polyesters[23, 24] or glyceride esters thermosets.[25] In a recent work, we reported on

the synthesis of novel mercapto-PCL bearing thiol side groups, which was cross-linked either via redox reaction, with formation of disulfide reversible bonds, or thermal thiol-ene reaction to yield a thioether-containing degradable network.[26] In a similar way, thiol-yne reactions can also be used for cross-linking of networks with the advantage that two equivalents of thiol per alkyne group can potentially react.[27] This particularity yields highly cross-linked structures compared to homologous thiol-ene systems, with an impact in thermomechanical properties.[28] However, thiol-yne cross-linking was mainly restricted to non-degradable networks[27, 29-32] except a few examples including thiol-yne cross-linking of aliphatic hyperbranched polyesters[33] or hetero-telechelic α-thiol ω-yne PCL.[34]

In the present work we therefore wish to report on the preparation of degradable networks prepared by thiol-yne photo cross-linking of an easily prepared PCL bearing multiple alkyne groups on its skeleton. The synthesis of this alkyne-PCL by post-polymerization modification is first presented. The photo cross-linking of blends of alkyne-PCL, PCL and thiols is then evaluated. Finally, the mechanical properties and biocompatibility of the resulting networks are discussed.

2. Materials and methods

2.1. Materials

PCL ($M_n \approx 32$ kg/mol), propargyl bromide (80 wt% in toluene), lithium diisopropylamide (2 M in THF/heptane/ethylbenzene), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP, >95%), 2,2-dimethoxy-2-phenylacetophenone or Irgacure® 651 (I651, 99%), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone or Irgacure® 2959 (I2959, 98%) were purchased from Sigma-Aldrich (St-Quentin Fallavier, France).

NH_4Cl (> 99%) was obtained from Acros Organics (Noisy-le-Grand, France), technical grade $MgSO_4$ from Carlo Erba (Val de Reuil, France), methanol (≥99.8%), dichloromethane (DCM, ≥99.9%) and anhydrous tetrahydrofuran (THF, ≥99.9%) from Sigma-Aldrich (St-Quentin Fallavier, France). THF was distilled on benzophenone/sodium until a deep blue color was obtained.

2.2. Characterizations

The number average molecular weight (M_n) and the dispersity ($Đ$) of the polymers were determined by size exclusion chromatography (SEC) using a Viscotek GPCMax autosampler system fitted two Viscotek LT5000L Mixed Medium columns (300×7.8 mm), a Viscotek VE 3580 RI detector. The mobile phase was THF at 1 mL/min flow at 30°C. Typically, the polymer (10 mg) was dissolved in THF (2 mL) and the resulting solution was filtered through a 0.45 µm Millipore filter before injection of 20 µL of filtered solution. M_n was expressed according to calibration using Polystyrene standards.

^{1}H NMR spectra were recorded at room temperature using an AMX300 Bruker spectrometer operating at 300 MHz. Deuterated chloroform was used as solvent, chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS). Diffusion ordered spectroscopy (DOSY) experiments were performed with a Bruker Avance III spectrometer operating at 600 MHz, using deuterated dimethyl sulfoxide (DMSO) as solvent.

The thermal properties of the polymers were characterized by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC measurements were carried out under nitrogen on a Perkin Elmer Instrument DSC 6000 Thermal Analyzer. Samples were submitted to a first heating scan to 150 °C (10 °C.min^{-1}) followed by a cooling (10 °C.min^{-1} from 150 °C to 100 °C, and 5 °C.min^{-1} from 100 °C to -65 °C) and a second heating scan to

180 °C (10 °C.min^{-1}). Glass transition temperature (T_g), crystallization temperature (T_c), melting temperature (T_m) and melting enthalpy (ΔH_m) were measured on the second heating ramp. TGA thermograms were obtained under a nitrogen flow (20 mL/min) on a Perkin Elmer Instrument TGA 6 at a scanning rate of 10 °C.min^{-1} from 30 to 400 °C.

Mechanical properties of the polymers were determined by performing tensile mechanical tests on plate samples (≈ 30 × 10 × 0.5 mm) at 37 °C on an Instron 4444 testing device at a crosshead speed rate of 5 mm/min (each sample being loaded to failure). Each sample was analyzed in triplicate and Young's modulus (E, MPa), stress at failure (σ_f, MPa), strain at failure (ε_f, %), yield stress (σ_y, MPa), and yield strain (ε_y, %) were expressed as the mean value of the three measurements. E was calculated using the initial linear portion of the stress/strain curves.

2.3. PCL propargylation

Typically, PCL (0.053 mol of CL unit, 6 g) was dissolved in anhydrous THF (300 mL) in a four-necked reactor equipped with a mechanical stirrer and kept at -50 °C under argon atmosphere. A solution of LDA (0.5 eq./CL unit, 13.2 mL) was injected with a syringe through a septum and the mixture was kept at -50 °C under stirring for 30 min. After this activation step, propargyl bromide (0.5 eq./CL unit, 2.8 mL) was added, the mixture was kept under stirring and raised to -30 °C. After 30 min, the reaction was stopped by addition of a saturated solution of NH_4Cl (100 mL) and pH was adjusted to 7 with 1M HCl. The polymer was extracted with DCM (3 × 150 mL). The combined organic phases were washed three times with distilled water (3 × 150 mL), dried on anhydrous $MgSO_4$ and filtered. After partial solvent evaporation under reduced pressure, the polymer was recovered by precipitation in

cold methanol and dried overnight under vacuum. The polymer was obtained with an average yield of 79 %.

^{1}H NMR : (300 MHz; $CDCl_3$) : δ (ppm) = 4.0 (2H, C*H*$_2$-O), 3.4 (2H, C*H*$_2$-OH), 2.4-2.6 (1H, C(O)C*H*(CH_2-C≡CH) and 2H, C*H*$_2$-C≡CH), 2.3 (2H, C(O)C*H*$_2$), 2.0 (1H, C≡C*H*), 1.6 (4H, C(O)CH_2-C*H*$_2$-CH_2-C*H*$_2$-CH_2-O), 1.4 (2H, C(O)CH_2-CH_2-C*H*$_2$-CH_2-CH_2-O).

2.4. Propargylated PCL photo cross-linking

Typically, mixtures of non-modified PCL and propargylated PCL (PCL-yne) (1.2 g of polymer, PCL-yne weight ratios from 0 to 100%) were dissolved in DCM (10 mL) with PETMP (0.25 eq./ propargylated CL unit) as cross-linker. Depending on the films no photoinitiator ($cPCL_x$) or 0.5 wt% photoinitiators I651 (6mg) ($c_{651}PCL_x$) or I2959 (6 mg) ($c_{2959}PCL_x$) were also added. For illustration purpose the notation $c_{651}PCL_{75}$ represents networks cross-linked with I651 and with an initial polymer composition of 25% of PCL and 75% of PCL-yne. On the other hand c_xPCL_{100} represents all networks prepared only with PCL-yne, whatever the cross-linker. The solutions were poured in aluminum molds and let to dry slowly overnight under a hood and in the dark. The resulting films were further dried under vacuum for 24 h. The photo cross-linking reaction was carried out under UV irradiation (10 min/side) with a UV light system Dymax PC-2000 (75 mW/cm²).

2.5. Cross-linking evaluation

After UV irradiation, samples of elastomers films were cut (≈ 100 mg), precisely weighed (initial weight W_i) and placed in DCM (15 mL). After 24 h, the insoluble cross-linked fractions were removed from DCM and weighed (swelled weight W_s). After drying under

vacuum overnight, the samples were weighed again (dry cross-linked weight W_c). To evaluate the cross-linking efficiency, the gel fraction and the weight swelling ratios of the polymer samples were calculated according to the following equations:

$$\text{Gel fraction (\%)} = 100 \times (W_c/W_i)$$

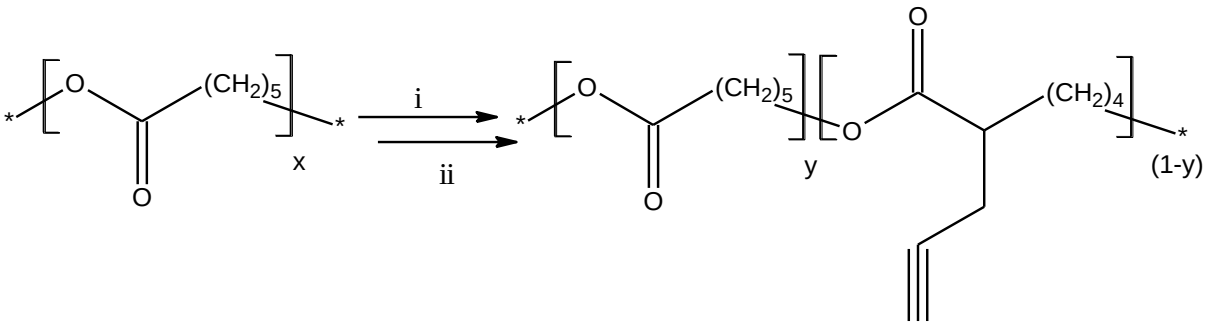
$$\text{Weight swelling ratios (\%)} = 100 \times (W_s/W_c).$$

Each result was expressed as the mean value of three measurements. The cross-linking was also evaluated by studying the thermal properties of the irradiated polymers before immersion in DCM.

3. Results

3.1. PCL propargylation

PCLs functionalized with alkyne functional groups have recently attracted attention as a result of the possibility to easily produce novel PCL-conjugates via click chemistry tools. In this regard, our group reported recently on the synthesis of α-propargyl-ε-caprolactone to yield propargylated PCLs by copolymerization with ε-caprolactone. This approach was advantageously used to prepare graft amphiphilic PCL copolymers and MRI-visible PCLs.[35, 36] Speaking of materials however, the synthesis of functional lactones does not appears as a convenient strategy as large amounts of products can barely be obtained. To overcome this limitation our group developed few years ago an anionic post-modification reaction that provided various functional PCLs with 5 to 15% of functional groups or polymeric side chains along the PCL backbone.[26, 37-41] With the aim to prepare cross-linked materials, the anionic post-polymerization modification strategy appears therefore as a sound choice to easily prepare PCL bearing multiple alkyne groups (PCL-yne).



Scheme 1. General reaction scheme for the synthesis of propargylated PCL (PCL-yne) with i) LDA (0.5 eq./CL unit), -50 °C, 30 min and ii) propargyl bromide (0.5 eq./CL unit), -30 °C, 30 min.

Propargyl groups were chosen to introduce alkyne functionalities on the PCL backbone (Scheme 1) and propargyl bromide was reacted with PCL under anionic chemical modification conditions. The parameters of the activation step with LDA (0.5 eq. ; - 50°C ; 30 min.) and of the subsequent substitution with propargyl bromide (0.5 eq. ; - 30°C ; 30 min.) have been optimized to obtain in a reproducible way PCL substituted with 8% of alkyne groups with respect to the CL units. In particular, the initial PCL concentration in the reaction medium has been optimized as lower substitutions were obtained with lower PCL concentrations (data not shown). Based on our experience, such a sensible dependence over the PCL concentration was not observed for other electrophiles. This is therefore our belief that with propargyl bromide, a higher concentration of PCL is required to favor the substitution reaction over the abstraction of the acidic alkyne proton by LDA. The polymer chemical structure was characterized by ^{1}H-NMR (ESI Figure S1). Typical signals of the propargyl group were found at 2.5 ppm, corresponding to the methylene proton and at 2.0 ppm, corresponding to the alkyne proton. A 8% substitution degree (SD) was calculated using

Equation (1) by comparison of the integrations of the well-defined resonance peak at 2.0 ppm with the integration of the resonance peak at 4.0 ppm, corresponding to a methylene of PCL.

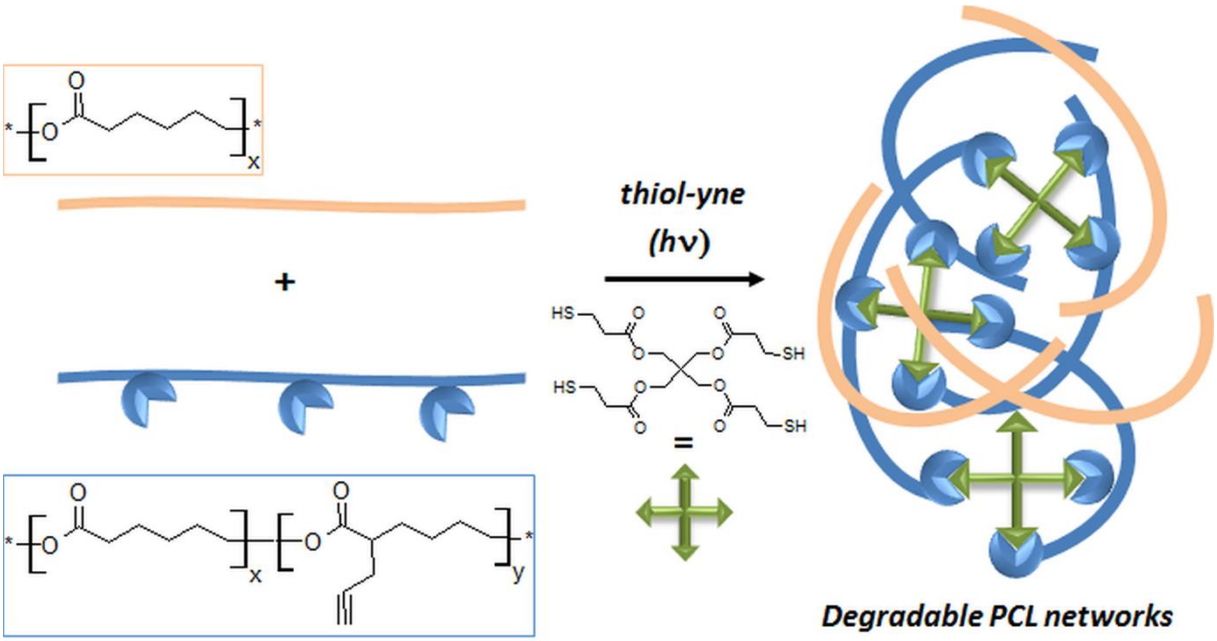
$$SD\ (\%) = I_{2ppm}/(I_{4ppm}/2)\times 100 \quad (1)$$

Molecular weight of PCL-yne was measured by SEC analysis in THF. PCL-yne with molecular weight Mn = 12 000 g/mol and Đ = 2.1 was obtained. These values were compared with the ones of the commercial PCL used as a starting material Mn = 32 000 g/mol and Đ = 1.9 (ESI Figure S2). The molecular weight decrease is classically observed with the anionic activation of polyester, as a consequence of hydrolysis and backbiting side reactions.[26, 41] In the present case, and although remarkable in terms of influence on the final molecular weight, this corresponded to less than 1% hydrolysis of the ester groups initially present in the polymer chain. Finally, it is of importance to note that starting from commercially available PCL, this one pot and time saving reaction allowed the preparation of ca. 5 g batches of PCL-yne in a single day, including reaction and purification. Synthesized PCL-yne was further used to produce photo cross-linked materials under UV activation.

3.2. Photo cross-linking

Cross-linking was obtained by photoradical-mediated thiol-yne click reaction between the alkyne pending groups of PCL-yne and the thiol groups of pentaerythritol tetrakis(3-mercaptopropionate) (PETMP). Films were prepared by the solvent evaporation method using mixtures of polymer and PETMP. I2959 and I651 Photoinitiators were also used in some films to evaluate their impact on the cross-linking efficiency. These photoinitiators were chosen due to their long track records in the field of biomaterials, especially I2959 which is known for its good biocompatibility.[42] Assays have been carried out with films prepared from mixtures of PCL-yne and neat PCL at different ratios. It is to note that the PCL-yne had low

filmogenic properties, which made tricky to obtain large homogenous films. This is related to the lower molecular weight of PCL-yne compared to neat PCL that probably does not allow good chain entanglement. All films were photo cross-linked under UV (10 min/side) with an intensity of 75 mW/cm² to yield mixed networks as shown in Scheme 2.



Scheme 2. Thiol-yne photocrosslinked PCL networks

Gel fraction and swelling were first evaluated. An insoluble fraction was obtained for all samples containing PCL-yne, the gel fraction increasing almost linearly with the proportion of PCL-yne (Figure 1). In the absence of photoinitiator gel fractions were slightly lower than with Irgacure®, whereas no significant differences were observed between I651 and I2959. The thiol-yne photo cross-linking efficiency with respect to the initial amount of PCL-yne was high for all samples. More in details, gel fractions were in the range 67% to 78% for samples containing only PCL-yne ($cPCL_{100}$, $c_{651}PCL_{100}$ and $c_{2959}PCL_{100}$) and 20% to ca. 25% for samples containing only 25% of PCL-yne. Photoinitiators led to higher cross-linking efficiencies and slightly denser network as discussed below. The impact of photoinitiators was

more clearly evidenced for networks having low contents of PCL-yne. A 25% higher efficiency was obtained in the presence of Irgacure® for the c_xPCL_{25} series ($c_{651}PCL_{25}$ and $c_{2959}PCL_{25}$ *vs.* $cPCL_{25}$) against 12% for the c_xPCL_{100} series ($c_{651}PCL_{100}$ and $c_{2959}PCL_{100}$ *vs.* $cPCL_{100}$).

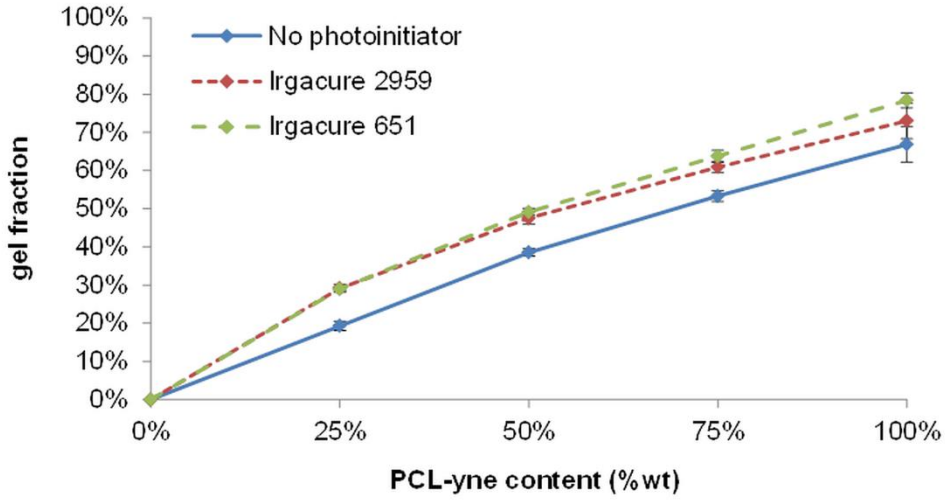


Figure 1. Gel fraction of UV-crosslinked PCL / propargylated PCL mixes with or without photoinitiator as a function of propargylated PCL content.

After removal of the free PCL chains, samples weight swelling ratios were characterized in DCM. The values obtained confirmed that although gel fractions were only slightly higher with Irgacure®, the resulting networks present a higher cross-linking density. This is clearly evidenced on c_xPCL_{25} samples with weight swelling ratios values of 1000% in the presence of photoinitiator ($c_{651}PCL_{25}$ and $c_{2959}PCL_{25}$) against 2100% without photoinitiator ($cPCL_{25}$) (Figure 2). As expected, the weight swelling ratios also decreased with increasing proportion of PCL-yne in the films, which indicates that neat PCL acted as a spacer between the alkyne groups, which led to networks of lower cross-linking density.

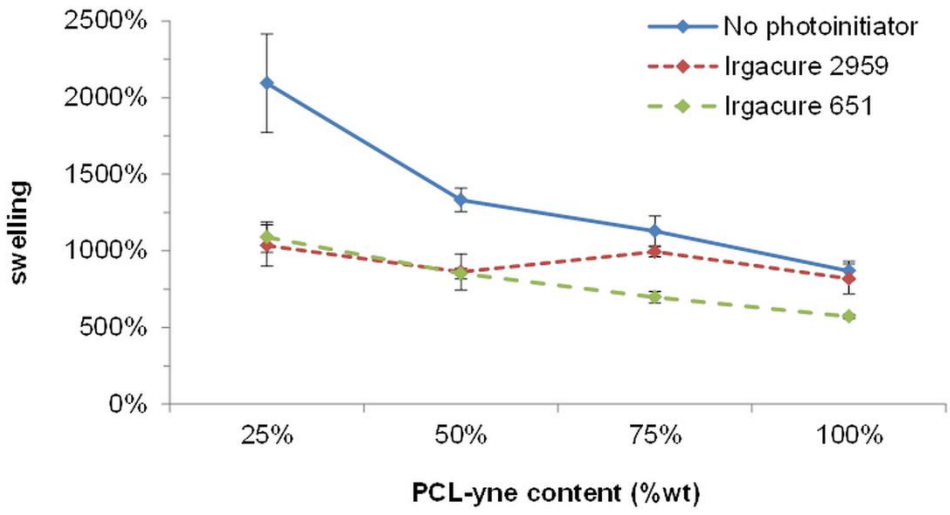


Figure 2. Swelling of UV-crosslinked PCL / propargylated PCL mixes with or without photoinitiator as a function of propargylated PCL content.

Finally, one should note that as residual free thiol groups of PETMP may potentially be present in the networks, disulfide bond formation could also be responsible to a limited extent for some cross-linking. In order to evaluate this point a control experiment has been done. More in details a $cPCL_{100}$ film was prepared and purified as described earlier. It was then swollen in DMSO under air atmosphere and heated at 70°C to favor the formation of disulfide bond.[26] Under these conditions, no difference in terms of gel fraction or mechanical properties was found with respect to non oxidized $cPCL_{100}$ (data not shown). If existing disulfide bond have therefore a negligible influence compared to thioether bonds on the networks formation and properties.

3.3. Thermal properties

Thermal properties analyses were conducted on networks obtained from PCL-yne ($cPCL_{100}$, $c_{651}PCL_{100}$ and $c_{2959}PCL_{100}$). TGA analyses have first been performed on the cross-linked and

non-cross-linked PCL-yne and compared to the commercial PCL (Figure 3). Thermograms showed that the presence of alkyne groups causes an early thermal degradation of PCL-yne compared to neat PCL with an onset temperature around 350°C against 420°C. After thiol-yne photo cross-linking in the presence of the PETMP, PCL-yne networks gained as expected thermal stability with respect to the starting PCL-yne as shown by the delayed thermal degradation occurring between 395°C and 410 °C, *i.e.* at temperatures similar to the ones obtained for the commercial PCL. No difference was observed for the networks cross-linked in the presence ($c_{651}PCL_{100}$ and $c_{2959}PCL_{100}$) or absence ($cPCL_{100}$) of photoinitiator.

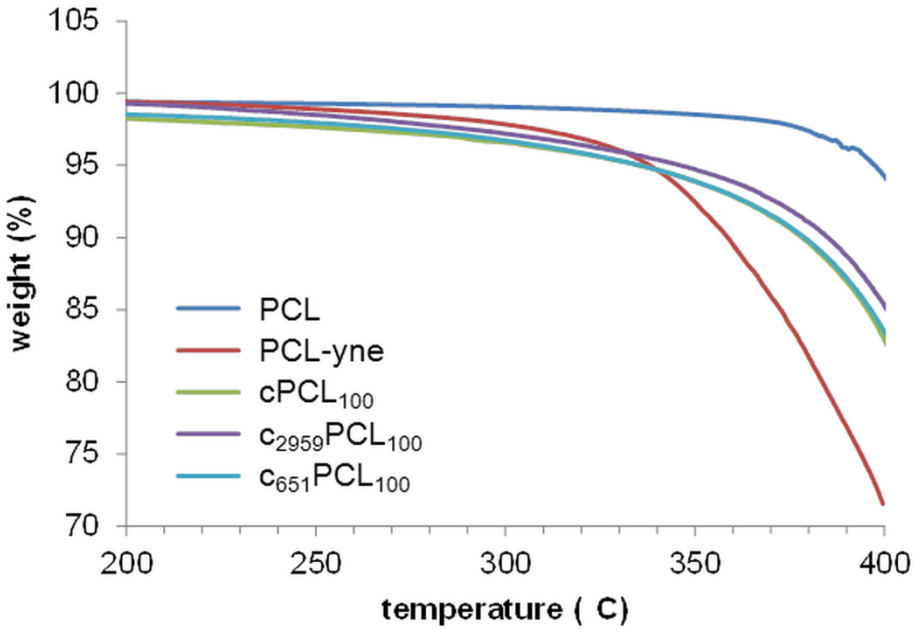


Figure 3. TGA thermograms of non-modified PCL, propargylated PCL and UV-crosslinked propargylated PCL with or without photoinitiator.

DSC analyses show that PCL is a semi-crystalline polyester (Figure 4). For commercially available PCL (Mn = 10000 g/mol) with a molecular weight close to the one of PCL-yne, a glass transition temperature (Tg) of -60°C, a melting temperature (Tm) of 65°C and a melting enthalpy ΔHm of 105 J/g were measured (data not shown). As a result of its lower molecular

weight and of the pending propargyl group, PCL-yne had lower Tm (45°C) and melting enthalpy ΔHm (70 J/g). After cross-linking, all networks were still semi-crystalline with ΔHm in the range 35-45 J/g. These lower values indicate a decrease of crystallinity, as expected for cross-linked polymers. However, the PCL segments between cross-links are still long enough to induce crystallization, PCL oligomers with low molecular weight (Mn= 1200 g/mol) being known to crystallize.[2] In the present case, this crystallization results in semi-crystalline materials. Again, like for swelling and gel fraction experiments, results show that a higher density of cross-linking is obtained with a photoinitiator, as shown by the lower Tm and ΔHm obtained with Irgacure®. They also confirm that I651 ($c_{651}PCL_{100,}$ Tm = 39.5°C, ΔHm = 38 J/g) is more efficient for thiol-yne curing of PCL-yne with PETMP than I2959 ($c_{2959}PCL_{100,}$Tm = 40.5°C, ΔHm = 43 J/g). Notably, all Tm were above the physiological temperature. Considering a melting enthalpy of 142 J/g for a 100% crystalline PCL,[43] crystallinities of PCL-yne, $cPCL_{100}$, $c_{651}PCL_{100}$ and $c_{2959}PCL_{100}$ have been calculated with values of 50%, 33%, 29% and 26%, respectively. Glass transition temperatures were measured and found equal for all networks with values in the range -8°C to -6°C (ESI Figure S3). As expected cross-linked networks had therefore higher Tg compared to PCL-yne whose Tg was not detectable in the experimental range.

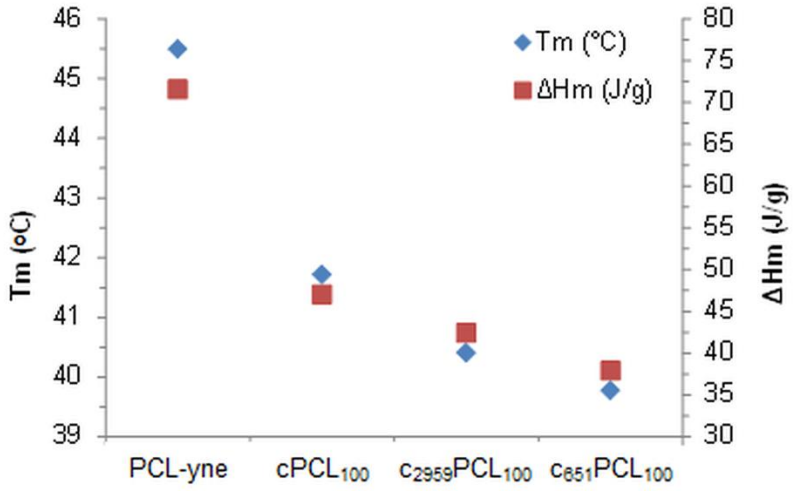


Figure 4. Melting temperature and melting enthalpy (measured by DSC) of propargylated PCL and UV-crosslinked propargylated PCL with or without photoinitiator.

3.4. Tensile Mechanical properties

Tensile mechanical tests have been carried out to evaluate the new PCL networks. As for gel fraction, tests were carried out on the cross-linked films prepared from mixtures of PCL-yne and neat PCL at different ratios. It was also compared to non-cross-linked PCL-yne films. Typical stress-strain curves are provided as Supplementary Information (ESI Figure S4). Cross-linking strongly improved the mechanical properties of PCL-yne as non-cross-linked polymer was barely testable under the conditions used, samples being broken even at low stresses with stress at failure $\sigma_f < 1$ MPa and Young's modulus $E < 20$ MPa (ESI Figure S4). In opposition, c_xPCL_{100} networks had higher mechanical properties with $\sigma_f \approx 4$ MPa, $\sigma_y \approx 2$ MPa and $E \approx 100$ MPa (Figure 5). Upon addition of neat PCL in the cross-linked networks, a general increase of Young's moduli and characteristic stresses was observed as a result of the higher molecular weight of the PCL. Typical values for c_xPCL_0 films were $\sigma_f \approx 14$ MPa, $\sigma_y \approx 5$ MPa and $E \approx 200$ MPa. For strain at failure (ε_f) and yield strain (ε_y), results were quite

different. Maximum values were obtained for films containing between 25% and 75% of PCL-yne (c_xPCL_{25}, c_xPCL_{50} and c_xPCL_{75}). For example, maximum ε_f values in the range 20% ($cPCL_{50}$) to 30% ($c_{651}PCL_{50}$ and $c_{2959}PCL_{50}$) were obtained, against 10% for pure PCL and c_xPCL_{100}. Regarding ε_y, the observed increase was more limited with a maximal improvement factor of 2 compared to neat PCL. Networks with intermediate polymer ratios showed therefore improved strains with respect to PCL and PCL-yne and moduli corresponding to soft tissues, for example ligaments.[44]

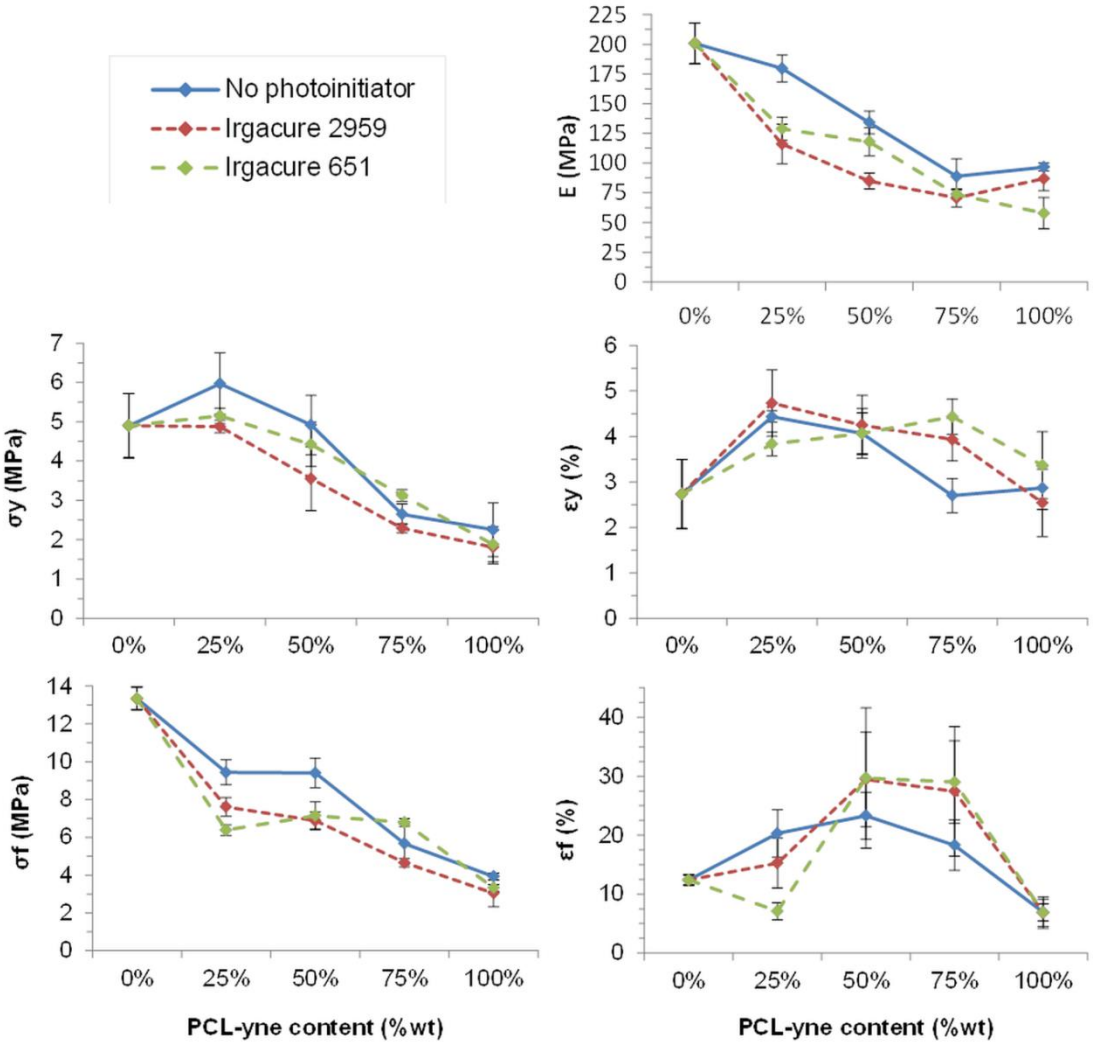


Figure 5. Mechanical tensile properties of UV-crosslinked PCL / propargylated PCL mixes with or without photoinitiator as a function of propargylated PCL content.

3.5. Networks Biocompatibility

Residual photoinitiators and cross-linkers in the polymer networks can be a problem for biomedical applications. In the present case, although PCL is well known for its biocompatibility, the potential toxicity of the cross-linked materials containing PETMP and Irgacure® residues had to be assessed. This was achieved by seeding L929 fibroblasts on sterilized samples and assessing the cell viability over a 10 days period. Behavior of the cells on the networks was compared to their behavior when cultured on TCPS control plates. Results are shown in Figure 6. L929 fibroblasts adhere and proliferate on all materials. Similar level of cell viability was observed for all networks, with no statistical differences in cell number in the presence or absence of photoinitiator during the curing. In particular, no difference was found between I651 and I2959 although the latter is generally recognized as being more biocompatible. In addition, for all samples the number of cells present on the surface of the network at different time points was between 60% and 75% of the one observed on TCPS control plates and cell proliferation steadily increased with time, which demonstrated the good biocompatibility of these materials.

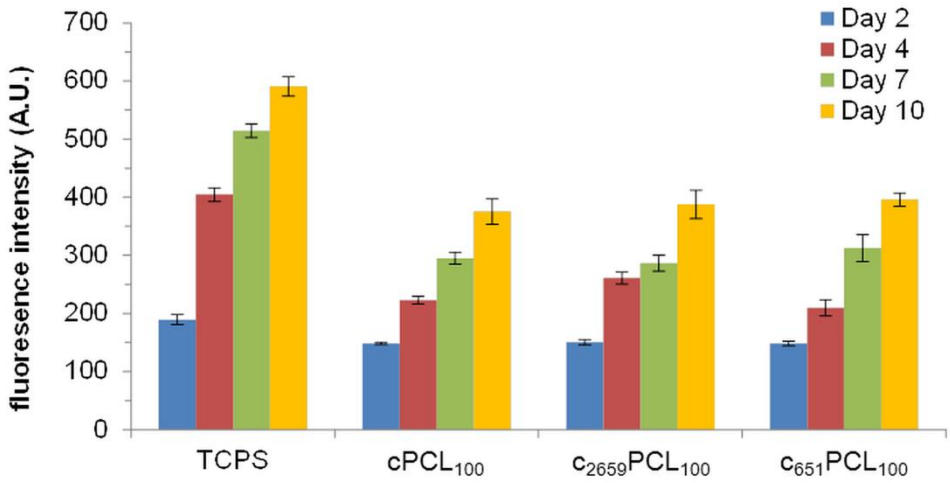


Figure 6. In vitro proliferation of L929 cells on TCPS positive control and UV-crosslinked propargylated PCL with or without photoinitiator.

Conclusion

Degradable cross-linked biomaterials were easily prepared via thiol-yne photochemistry between an alkyne functional PCL and PETMP. For the first time, a one pot and timely efficient post-polymerization modification reaction of PCL was reported to yield the multifunctional PCL-yne. Pure PCL-yne and mixtures of PCL-yne and PCL were cross-linked under UV via photoradical thiol-yne reaction in short times and with high efficiencies. These novel biocompatible networks have mechanical properties close to the ones of soft tissues, which is an open route for the design of biomedical scaffolds.

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