irradiated at 100 °C and 100 W for 30 min. After precipitating the reaction mixture with 50 mL of methanol and washing three times with 10 mL of CH_2Cl_2, the final product dextran-g-(PCL-b-PEG) was obtained.

Results and discussion

As shown in Scheme 1, dextran (M_w 500,000) was propargylated with propargyl bromide (PgBr) in DMSO in the presence of sodium hydroxide (NaOH) The resultant degree of substitution (DS) was 76 alkyne groups/dextran (calculated by ^1H NMR spectrum). A higher DS would lead to insoluble products, presumably due to the trimerization of PgBr. PEG-b-PCL was synthesized by ROP method. The feed ratio of [CL] to [OH] of mPEG was 90:1 and the PCL chains had an average degree of polymerization (DP) of 81 (calculated by ^1H NMR spectrum). Its end OH group was activated by MesCl and replaced by an azido group in DMF to obtain PEG-b-PCL-N_3.
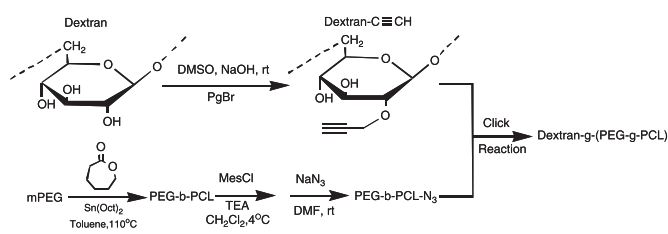
Scheme 1. Synthetic route of dextran-g-(PCL-b-PEG).

The FT-IR spectrum of PEG-b-PCL-N_3 (Fig. 1a) showed a significant band at 2096 cm^{-1} for the azide moiety. Through "click" chemistry, dextran-C≡CH and PEG-b-PCL-N_3 reacted in the presence of $CuSO_4$/ sodium ascorbate in DMSO by microwave assisted synthesis. From the FT-IR spectrum (Fig. 1b), it can be found that the typical peak of azide groups at around 2096 cm^{-1} disappeared compared to the IR spectrum of PEG-b-PCL-N_3 (Fig. 1a) before the click reaction. The typical peak of ester bonds in PEG-b-PCL at 1733 cm^{-1} also appeared in the reaction product, which indicated the success of the click reaction. The grafting degree was 34 PEG-b-PCL/dextran (calculated by ^1H NMR spectrum).
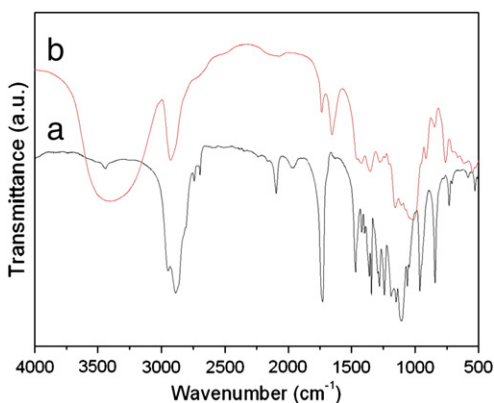
Fig. 1. FT-IR spectra of (a) PEG-b-PCL-N_3; (b) Dextran-g-(PCL-b-PEG).

Using a nanoprecipitation method, these graft copolymer with amphiphilic side chains self-assembled into nanoparticles in aqueous solution easily. The morphology of the self-assembled nanoparticles was investigated by TEM. The result (Fig. 2) shows that these graft copolymers self-assembled into spherical micelles with an average diameter of less than 50 nm. As shown in Scheme 2, these micelles are composed of three layers: PCL as core plus dextran as shell plus PEG on the surface towards the water phase. The dextran backbone plays a role
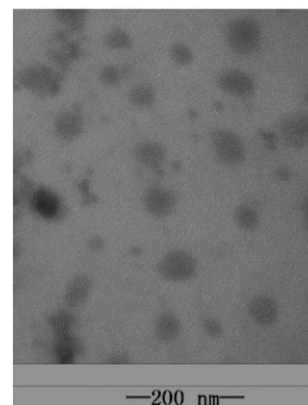
Fig. 2. TEM image of self-assembled dextran-g-(PCL-b-PEG) micelles.

of cross-linker for PCL segments. Compared with PCL-PEG micelles, dextran-g-(PCL-b-PEG) micelles would show enhanced stability. Also PEG on the surface will increase their residence time in systemic circulation. These properties make these micelles suitable for targeted drug delivery through size-mediated accumulation. Further surface modification may lead to active targeting of the micelles to tumors.
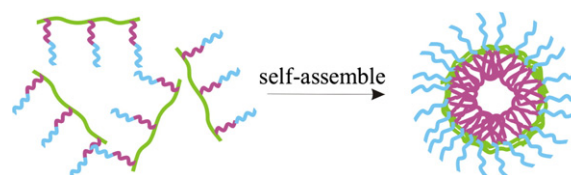
Scheme 2. Schematic structure of dextran-g-(PCL-b-PEG) micelles.

Conclusion

We have demonstrated the synthesis of dextran-g-(PCL-b-PEG) by combination of ROP and click chemistry. These graft copolymers with amphiphilic side chains were completely biodegradable and biocompatible. These polymers also can self-assemble into spherical micelles with a diameter of less than 50 nm. Future studies on their drug loading and release are undertaken and related results will be reported elsewhere.

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Biodegradable, *in situ* forming poly(ethylene glycol)-poly(lactide) hydrogels by Michael addition chemistry

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Abstract summary

Biodegradable, chemically crosslinked poly(ethylene glycol)-poly
(lactide) (PEG-PLA) hydrogels were synthesized by a Michael
addition reaction between acrylated PEG-PLA star block copolymers
and thiol-terminated multi-arm PEG. A gelation time of 1 min was
achieved when the macromonomers were crosslinked in PBS and the
resulting hydrogels exhibited a storage modulus of 25 kPa. *In vitro*
degradation experiments revealed that the hydrogels lost 30% of
polymer mass in 30 days as a result of the hydrolysis of PLA chains in
the block copolymers.

Keywords: PEG, PLA, Chemically crosslinked, Hydrogel

Introduction

Hydrogels are polymer networks which are able to swell
considerably and retain large amounts of water in their swollen
structures [1]. Biodegradable PEG-PLA type hydrogels generally
exhibit excellent biocompatibility and are accordingly of interest for
biomedical applications such as tissue engineering and systems for
controlled delivery of biologically active agents. *In situ* forming
hydrogels offer several advantages over systems that have to be
formed into their final shape before implantation: there is no need for
surgical procedures, their initially flowing nature ensures proper
shape adaptation as well as a good fit with surrounding tissue, and
biologically active species such as cells or growth factors can be
incorporated homogeneously in the hydrogel by simple mixing with
the uncrosslinked polymer solution [2]. Both chemical and physical
methods can be used to create the three-dimensional networks
in situ. In general, physically crosslinked thermosensitive PEG-PLA
hydrogels are mechanically weak. In this study, we describe a
hydrogel system that forms rapidly by a Michael addition reaction
between multi-arm PEG-thiols and acrylate-terminated PEG-PLA star
block copolymers.

Experimental methods

Polymer synthesis. 8-armed poly(ethylene glycol)-poly(l-lactide)
star block copolymer was synthesized by ring opening polymerization
of l-lactide in toluene at 110 °C. Amine-terminated 8-armed star PEG
($M_{n, NMR}$ 23800 g/mol) and stannous octoate were used as initiator and
catalyst. The PEG-PLA star block copolymers were functionalized
with acrylate moieties according to a previously reported method [3].
8-armed PEG-thiol was synthesized starting from PEG-(OH)$_8$ ($M_{n, NMR}$ =
23700 g/mol) following a three-step procedure analogous to that
described by Goessl et al. for linear hydroxyl-terminated PEGs [4].

Hydrogel preparation. Macromonomer solutions were prepared by
dissolving PEG-(SH)$_8$ and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR in PBS (pH 7.4) at
a concentration of 5 w/v%. The macromonomer solutions were mixed
and the Michael addition reaction was allowed to proceed for 48 h under
gentle shaking in an inert atmosphere, resulting in the formation of a
hydrogel.

Rheology. To determine the kinetics of gel formation, separate
PEG-(SH)$_8$ and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR macromonomer solu-
tions in PBS were mixed, homogenized and quickly applied to the
rheometer. A layer of oil was applied on top of the gels to prevent
water evaporation. Experiments were performed at 25 °C, using a flat
plate measuring geometry (diameter 25 mm, gap 0.3 mm), a strain of
1% and a frequency of 1 Hz.

Degradation. Freshly prepared hydrogel samples were dried, their
initial weight W_0 was determined and samples were immersed in
PBS at 37 °C. At regular times, samples were taken out and their mass
in swollen state (W_s) was measured after wiping the surface with
tissue paper. The degree of swelling during degradation was
calculated from: degree of swelling = $(W_s - W_0)/W_0 \cdot 100\%$. Subse-
quently, the samples were allowed to dry overnight to yield the dry
weight after degradation (W_D). The polymer mass during degrada-
tion was calculated as: polymer mass = $(W_D/W_0) \cdot 100\%$.

Results and discussion

PEG-(NHCO)-(PLLA$_{11}$)$_8$ star block copolymer was prepared by ring
opening polymerization of l-lactide initiated by PEG-(NH$_2$)$_8$ in the
presence of stannous octoate as a catalyst. As compared with PEG-
(OCO)-(PLA)$_8$ star block copolymers with an ester linkage between
PEG and PLA, PEG-(NHCO)-(PLA)$_8$ polymers form hydrogels at lower
polymer concentrations and show higher storage moduli and
prolonged stability *in vitro* [5]. Nonetheless, physically crosslinked
hydrogels are not sufficiently stable for long-term biomedical
applications such as cell-supportive matrices for tissue engineering.
Acrylation of PEG-based polymers has proven an efficient way to
prepare chemically crosslinked hydrogels via a Michael addition
reaction [3]. The PEG-(NHCO)-(PLLA$_{11}$)$_8$ star block copolymer was
end group functionalized using acryloyl chloride in dichloromethane.
The degree of acrylation as calculated from ¹H NMR spectra appeared
higher than 90%. The acrylate-reactive PEG-(SH)$_8$ star polymer was
produced in high yield starting from PEG-(OH)$_8$. ¹H NMR spectro-
scopy revealed almost quantitative thiol functionalization.

Mixing PEG-(SH)$_8$ and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR macromono-
mer solutions resulted in the formation of yellow and transparent
PEG-PLA hydrogels. Ellmann's reagent was used to determine the
number of free thiol groups after network formation. It was found
that after 48 h, only 1% of the original thiol groups remained,
indicating that networks with a high crosslink density were obtained
with little dangling polymer chains.

Gel formation kinetics were studied by monitoring the storage
modulus (G′) and loss modulus (G″) in time after mixing PEG-(SH)$_8$
and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR macromonomer solutions (Fig. 1).
The crossover point of G′ and G″, which can be regarded as the gel
point, was observed after 1 min. The storage modulus levels off after
20 minutes and eventually reaches a value of 25 kPa. These data
show that mixing PEG-(SH)$_8$ and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR
macromonomer solutions results in the *in situ* formation of a
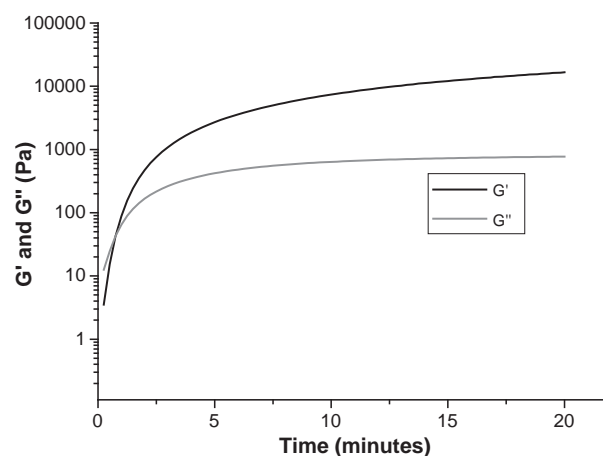chemically crosslinked PEG-PLA hydrogel with robust mechanical
properties.

Fig. 1. Storage modulus (G′) and loss modulus (G″) versus time after mixing PEG-(SH)$_8$
and PEG-(NHCO)-(PLLA$_{11}$)$_8$-ACR macromonomer solutions at 25 °C.

Fig. 2 shows the polymer mass loss over time from chemically crosslinked PEG-PLA hydrogels, incubated in PBS at 37 °C. No polymer mass loss is observed during the first 10 days. In the following 20 days the networks loose approximately 30% of polymer mass, most likely as a result of hydrolysis of ester groups in the PLA domains. The degradation of the networks is accompanied by an increase in swelling (Fig. 2). The ongoing swelling is probably due to PLA degradation, upon which chemical and physical crosslinks are lost, resulting in a less densely crosslinked network. The results indicate that the chemically crosslinked PEG-PLA hydrogels possess excellent *in vitro* stability.
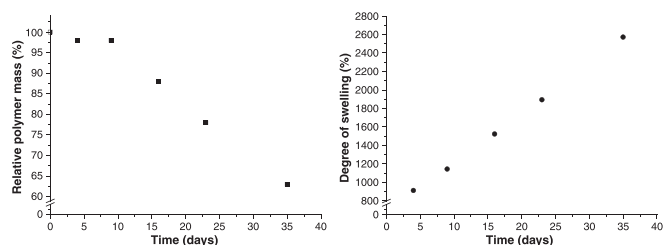

Fig. 2. Polymer mass (left) and degree of swelling (right) versus time for chemically crosslinked PEG-PLA hydrogels in PBS at 37 °C.

Conclusion

Chemically crosslinked PEG-PLA hydrogels were prepared by a Michael addition reaction between PEG-$(SH)_8$ and PEG-(NHCO)-$(PLLA_{11})_8$-ACR macromonomers in PBS. Rheological measurements showed that the hydrogels form *in situ* and possess robust mechanical properties. Degradation studies indicated that the hydrogels exhibit a good *in vitro* stability with 30% mass loss after 30 days. These PEG-PLA hydrogels hold promise for use as injectable systems for biomedical applications such as the controlled delivery of active agents.

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The investigation of cell adhesion on nano-patterned biointerfaces of block copolymer films by reactive microcontact printing approach

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Abstract summary

A new reactive microcontact printing strategy to fabricate well-defined functional micro- and nanostructured biointerfaces is introduced. Hepg2 liver cells were observed to spread in highly spatially directed pronounced elongated shapes on the nanopatterned platforms in a controlled way.

Keywords: Polymer, Biointerfaces, Pattering, Cell culture

Introduction

Patterned biofunctional interfacial architectures are currently at the focal point of sensor and cell biology research [1]. For a wide range of possible applications in the life sciences, in particular, the investigation of controlled cell-surface interactions, (bio)chemical patterning on multiple length scales down to the sub-100 nm level is required. The relevant distances and length scales of ligand clustering in multivalent recognition and those in protein clustering in focal adhesion of certain types of cells on the one hand [2], and the size of biological entities such as bacteria or cells span an enormous range [3]. In this communication we introduce a new reactive microcontact printing (μCP) strategy to fabricate well-defined functional micro- and nanostructured biointerfaces. Central to our new approach is the exploitation of selective surface chemistry on a reactive block copolymer film by using a volatile and highly diffusive reactant (trifluoroacetic acid) delivered via an elastomeric stamp (Fig. 1) [4].

Experimental methods

Materials. Polystyrene-*block*-poly(*tert*-butyl acrylate) (PS690-*b*-P*t*BA1210) diblock copolymers were purchased from Polymer Source Company (Dorval, Canada). Amino end-labeled PEG (PEG5000-NH_2) was purchased from Nektar UK Company, fluoresceinamine and fibronectin were purchased from Molecular Probes, Inc. (Shanghai). Thin films were prepared as reported previously by spin-coating filtered polymer solutions in toluene [5] and reactive microcontact printing on the films according to a procedure published earlier [6].

Characterization. Fluorescence microscopy images were recorded at room temperature on a Zeiss LSM 510 confocal laser scanning microscope. Optical microscopy was performed using an Olympus BX 60 (standard setup). The contact-mode AFM measurements were carried out with a NanoScope III multimode AFM [7].

Cell culture. Liver cells Hepg2 were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% fetal calf serum (FCS), 5% horse serum and 2 mM L-glutamine at 5% CO2.

Results and discussion

The PS690-*b*-P*t*BA1210 block copolymer film platform utilized in this study comprises reactive *tert*-butyl ester moieties at the film surface (skin layer thickness ca. 8 nm) and microphase-separated glassy polystyrene domains in the film interior that provide excellent thermal and processing stability. The volatile and highly diffusive reactant TFA is delivered locally in the (sub-)microcontacts between an elastomeric stamp and a reactive block copolymer film (Fig. 1).
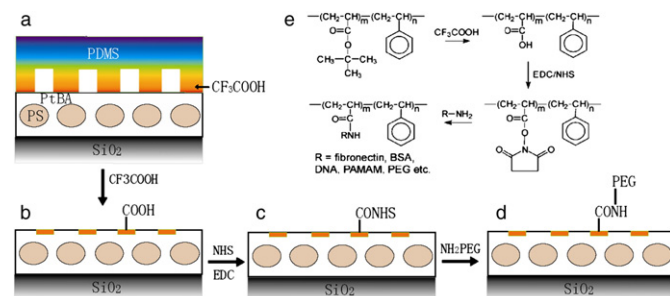

Fig. 1. Schematic of new reactive microcontact printing strategy and functionalization on block copolymer films.