Precise control of drug loading and release of an NSAID–polymer conjugate for long term osteoarthritic intra-articular drug delivery†

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A facile synthesis method of polymer diclofenac conjugates (PDCs) based on biocompatible polyurethane chemistry that provides a high drug load, and offers a high degree of control over diclofenac (DCF) release kinetics is described. DCF incorporating monomer was reacted with ethyl-L-lysine diisocyanate (ELDI) and different amounts of polyethylene glycol (PEG) in a one-step synthesis to yield polymers with pendant diclofenac distributed along the backbone. By adjusting the co-monomers feed ratio, the drug loading could be tailored accordingly to give DCF loading of up to 38 w/w%. The release rate could also be controlled easily by changing the amount of PEG in the backbone. Above 10 w/w% of PEG, the in vitro DCF release studies in physiological conditions showed an apparent zero-order profile without an initial burst effect for up to 120 days. The PDCs described may be suitable for long-term intra-articular (IA) delivery for the treatment of osteoarthritis (OA).

A sustained course of non-steroidal anti-inflammatory drugs (NSAIDs) is often employed in the management of osteoarthritis (OA).1 However, the long term use of NSAIDs can lead to severe systemic side effects such as gastrointestinal ulceration or hemorrhage, increased risk of heart attack, renal failure and depression.2 Due to the localized nature of OA, a site specific intra-articular (IA) drug injection has become an attractive approach to administer the therapeutic agents directly into the joint in an effort to minimize systemic side effects while concentrating drug delivery at the site of disease.3 However, the long term use of NSAIDs can lead to severe systemic side effects such as gastrointestinal ulceration or hemorrhage, increased risk of heart attack, renal failure and depression.2 Due to the localized nature of OA, a site specific intra-articular (IA) drug injection has become an attractive approach to administer the therapeutic agents directly into the joint in an effort to minimize systemic side effects while concentrating drug delivery at the site of disease.3

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prolonged period (e.g., for OA treatment). The synthesis method employed involves a step-growth polymerization in which a new diclofenac-incorporating diol monomer is used with a corresponding diisocyanate co-monomer in the polyurethane synthesis. This method allows for facile control of the drug loading and drug release kinetics through simple adjustment of the ratio of the diclofenac-incorporating diol monomer to other diol co-monomers (e.g. polyethylene glycol, PEG).

Wang et al.\textsuperscript{30} have extensively studied the hydrolysis of two diclofenac incorporating monomers, diclofenac-(p-hydroxybenzoate)-2-monoglyceride (DCF-PHB-MG) (2) and diclofenac-monoglycerides (DCF-MG) to show that under physiological conditions, the aryl ester construct (DCF-PHB-MG (2)) had a more rapid rate of hydrolysis compared to the alkyl ester, DCF-MG. They also showed two products arose directly from the hydrolysis of DCF-PHB-MG and DCF-MG and identified them as diclofenac and its cyclic lactam (an unwanted side-product). In view of the results of Wang, et al.\textsuperscript{30} initial polymerizations were carried out to test the likelihood of releasing DCF from PDCs produced with these DCF monomers. In contrast however, the PDCs obtained from DCF-PHB-MG as the incorporating monomer successfully released DCF under physiological conditions with almost no lactam observed. Furthermore, once incorporated into the polymers, our unpublished studies also showed that little or no diclofenac was released from the polymer with the alkyl ester linkage (DCF-MG) as the incorporating monomer. This result suggests that attaching the prodrug onto a polymeric delivery vehicle is able to protect the chemical fidelity of the therapeutic cargo. Based on this finding, DCF-PHB-MG (2) was chosen as the preferred diclofenac incorporating monomer for the subsequent polymerizations.

In the reported example, diclofenac (DCF, 1) was converted into a diclofenac-(p-hydroxybenzoate)-2-monoglyceride (DCF-PHB-MG, 2) as a polymerizable diol with DCF connected via a labile aryl ester linker (Scheme 1).\textsuperscript{31} The DCF–polymer conjugates were synthesized in a one-pot synthesis by reacting ethyl-L-lysine diisocyanate (ELDI) with different ratios of the diol co-monomers, polyethylene glycol (PEG) and DCF-PHB-MG (2) in the presence of a catalyst, dibutyltin dilaurate (DBTDL) to give linear polyurethane with pendent drugs distributed along the backbone (Scheme 2). These monomers were chosen because of their known biocompatibility and their ability to biodegrade into non-toxic by-products.\textsuperscript{28} The stoichiometric ratio was adjusted such that there was an equimolar ratio of isocyanates to the total hydroxyl groups of the diol monomers; PEG and DCF-PHB-MG (2) to enable the highest molecular weight possible to be achieved within the thermodynamic and kinetic constraints of polyurethane synthesis.\textsuperscript{32,33}

In order to investigate the versatility and tuneability of the drug loading and release rate of the PDC, a series of DCF polyurethanes with various DCF and PEG content have been synthesized. Three different PEG lengths were chosen as the co-monomers: PEG200, PEG400 and PEG1000. PEG MWs higher than 1 kDa were not considered here as it would lower the polymer drug loading dramatically, making this polymer unsuitable for drug delivery applications. The different feed ratios, the molecular weight distribution and stoichiometric ratio of the final polymer composition are presented in Table S1, ESI.\textdagger

For each PEG length, several stoichiometric ratios were systematically used in order to study the effect of the PEG content on the drug release rate. In addition, the stoichiometric ratio was adjusted to give polymers with a similar range of theoretical PEG content (w/w% weight of PEG per weight of polymer) regardless of the PEG length used. For example DCF P3 would have similar PEG w/w% content (11.8 w/w% PEG200) as P9 (10.3 w/w% PEG400) and P14 (10.4 w/w% PEG1000). This was done to determine whether there was a direct relationship between the PEG content (w/w%) and the release rate irrespective of PEG molecular weight. DCF polyurethanes without PEG was also synthesized to observe the effect on the release profile when no PEG is present in the backbone. The GPC analysis of the polymers showed they had average molecular weights within the
Further confirmation of polymer formation was established by means of FT-IR analysis which revealed the disappearance of the isocyanate peak from ELDI (~2245 cm⁻¹) and appearance of the –NH in the urethane linkage (~3310 cm⁻¹) from the backbone of the PDC, represented by polymer P4 (Fig. 1A). A typical ¹H NMR spectrum of the PDCs synthesized is shown in Fig. 1B. ¹H NMR spectroscopy was used to determine the stoichiometric ratio of the final composition of the polymer from ¹H NMR spectra can be used to determine the composition of the final polymers (in mol%) by comparing the integration ratio of the methylene peak of the PEG backbone (peak 1–4, δ 3.55–3.75 ppm), the aromatic proton of the DCF (peak 23, δ 6.57 ppm) and the methylene proton on the ELDI side chain (peak 12, δ 3.13 ppm). The integration ratio from the ¹H NMR spectra showed that the final polymer composition of all the polymers was in good agreement (~3%) with the molar feed ratio of the starting materials.

The stoichiometric ratio of the final composition of the polymer from ¹H NMR spectra can be used to determine the total DCF loading (w/w% DCF only, not DCF–PHB–MG per weight of polymer) and revealed that the loading could easily be tuned to give a range from 11.6 w/w% to 38.2 w/w% DCF per weight of polymer. To corroborate the total drug loading from ¹H NMR experiments, an available drug assay was performed using HPLC-UV analysis. Briefly, the polymers were digested using 5.0 M HCl to cleave all the pendent DCF from the polymers. Under these acidic conditions diclofenac was converted into its stable form, diclofenac lactam (1-(2,6-dichlorophenyl)-indolin-2-one) (Fig. S1, ESI†) the concentration of which was determined against a calibration plot of diclofenac lactam concentration against absorbance, which in turn enables the total available DCF to be calculated. As seen from Table S1 (ESI†) the available drug assay results from ¹H NMR experiments generally gives a higher drug loading compared to the HPLC (~8.0 w/w%). This was attributed to the polymeric nature of the PDC, which caused line broadenings in the ¹H NMR spectra (Fig. 1), reducing the accuracy of the peak integration and may lead to an overestimation of the drug loading.

These results showed that the polymerization method allows for precise control over DCF incorporation by simply changing the amount of the DCF–PHB–MG (2) and the PHB monomer used in the feed material. This is difficult to achieve through physical encapsulation or post-polymerization functionalization. The in vitro release studies of the DCF polymer with PEG (P1–P18) and without PEG (P0) in the backbone were performed by immersing the polymers in PBS buffer (pH = 7.4) at 37 °C with gentle stirring, and the amount of the DCF released was measured by means of HPLC-UV. The cumulative release of DCF (i.e. the mass DCF released/mass DCF available, wt% (µg µg⁻¹)) over time is shown in Fig. 2. DCF polymers without any PEG in the backbone (P0) displayed a lag phase of around 115 days before starting to release DCF and F released DCF very slowly (<3 wt% of the available DCF released in 135 days) (Fig. 2A). ELDI and DCF–PHB–MG (2) are quite hydrophobic, and therefore the observed lag phase could be attributed to water penetration barrier as a result of the hydrophobic starting materials. In order to reduce the hydrophobicity, the polymer was blended with 70 wt% PEG3000 (P0–blended) by dissolving in DCM and stirring the mixture for one hour before drying in vacuo. PEG3000 was preferred over lower molecular weight PEGs as the diluent in the blended experiments due to its comparable physical properties to the DPC. The addition of PEG3000 eliminated the lag phase as the blended polymer started to release DCF almost immediately. However, the release rate is still very low, with only 8 wt% of the available DCF released after 115 days.

For the polymers having PEG covalently incorporated in the backbone, it appears that a minimum threshold of PEG exists below which there is a lag phase of up to 115 days. This threshold differs for each PEG length i.e. ≤12 mol% PEG200 (P2), ≤9 mol% PEG400 (P9) and ≤6 mol% PEG1000 (P16) (Fig. 2B–D), respectively. However, above this threshold the onset of the DCF release occurred almost immediately with a zero-order release profile and almost no burst release observed. The release rate was also much higher compared to the control P0-blended polymer with 70 wt% PEG3000. For polymers with little or no PEG covalently incorporated in the backbone, the DCF is likely to be distributed with a pendent moiety coincident with every lysine moiety along the backbone (Scheme 2).

![Fig. 1](image-url) (A) FT-IR spectra of ELDI, DCF–PHB–MG (2), PEG200 and representative PDC (P4) and (B) representative ¹H NMR spectrum (CDCl₃) of PDCs containing pendent diclofenac groups distributed along linear polyurethane backbone.
Such an architecture is likely to result in the formation of closely packed or high density hydrophobic pendant moieties distributed along the polymer backbone which we hypothesize facilitate strong hydrophobic interactions between polymer chains in polymer system. Consequently, these intermolecular interactions retarded water penetration and significantly slows the rate of hydrolysis of the pendent aryl ester that links DCF to the polymer, resulting in the observed lag phase. The blending experiment described above shows that adding PEG as a mixture could disturb this hydrophobic interaction between polymer chains which was demonstrated by the concomitant elimination of lag phase. However, high density hydrophobic DCF pendant moieties still exist along the polymer backbone which continue to act as a barrier for water to hydrolyze the labile aryl ester linker. Consequently, even though the lag phase was eliminated, the rate of DCF release remained slow. After extended period of exposure (i.e. lag phase), water eventually penetrates these hydrophobic regions to hydrolyze some of the pendent DCF, leaving behind pendent aryl alcohol with hydroxyl group suspended along the polymer backbone. This slowly reduces the hydrophobicity of the polymer, and enabled hydrolysis of the remaining labile aryl ester and accompanying release of the DCF. The observed increase in the cumulative DCF in vitro release rate, which interestingly assumes a zero-order release profile once the lag phase is over, concurs with the changing architecture hypothesis.

It can also be observed from Fig. 2 that when a sufficient amount of PEG was covalently incorporated into the polymer backbone, the onset of the DCF release in physiological buffer solution occurred almost immediately. We attribute this to the PEG units acting as spacers between the hydrophobic pendent DCF moieties and reducing the local density of the hydrophobic groups distributed along the polymer backbone. As a result, there is increased accessibility for water to the labile aryl ester immediately upon immersion in the release media. For those polymers without the lag phase, the DCF release showed a zero-order profile regardless of the incorporated PEG length used. The rate of DCF release was found to increase as the PEG content increased, and as a result the time to reach complete DCF release was reduced at higher mol% of PEG. For example, DCF was continuously released for up to 120 days when 18 mol% of PEG200 was incorporated in the backbone (P3) (PEG200 series, Fig. 2B). However, the release of DCF was more rapid when 40 mol% PEG200 (P6) was used, with all DCF found to be released within 50 days.

Similarly, in the PEG400 series the polymer with 12 mol% PEG400 (P10) reached a plateau after 150 days. When the PEG400 content was increased to 20 mol% (P12), complete DCF release was achieved after approximately 90 days, while at 25 mol% (P13), complete release was achieved after 79 days (Fig. 2C). In the PEG1000 series, when 8 mol% PEG1000 or more was used, the lag phase was eliminated. In this case the DCF release reached a plateau after 74 days (Fig. 2D). DCF release ranging from 50 days up to 120 days, simply by changing the PEG content of the polymers.

In order to elucidate the relationship between the PEG content (w/w%) and the average release rate of DCF, modified cumulative release graphs were plotted to exclude the lag phase on the polymers with low PEG content (Fig. S2, ESI†): this enabled the comparison of the average release rate over the zero-order (linear) region of the DCF cumulative release plot. The average release was calculated by taking the average of the normalized release of DCF per 10 mg of polymer per day (µg per 10 mg per day) until the release plateaued.

Fig. 2 Cumulative release of DCF in isotonic phosphate buffer (pH 7.4; 37 °C) from DCF–polymer containing no PEG (A) and various mol% of (B) PEG200, (C) PEG400 and (D) PEG1000 in the backbone.
Conclusions

In conclusion, we have demonstrated an effective method for the synthesis of polymer-diclofenac conjugates (PDCs) via polyurethane chemistry that provides both a sustained release of diclofenac with no burst effect and high drug loading. Moreover, the method allows for facile control of the drug loading and release kinetics simply by changing the feed ratio of the co-monomers. Application of this method allows the drug loading to be easily tailored by changing the feed ratio of the co-monomers, and give PDCs with up to 38 w/w% DCF loading. We have also demonstrated that the drug release kinetics could easily be altered by changing the amount of PEG co-monomers to release all the pendent DCF in a controlled manner from 50 days up to 120 days, depending on the amount of PEG used and the desired application/treatment period. The PDCs described may be suitable for long term intra-articular (IA) delivery for the treatment osteoarthritis (OA). Currently, some works are underway in testing the efficacy of the polymers in an in vivo validated animal model.

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