Biodegradable microcapsules designed *via* 'click' chemistry†

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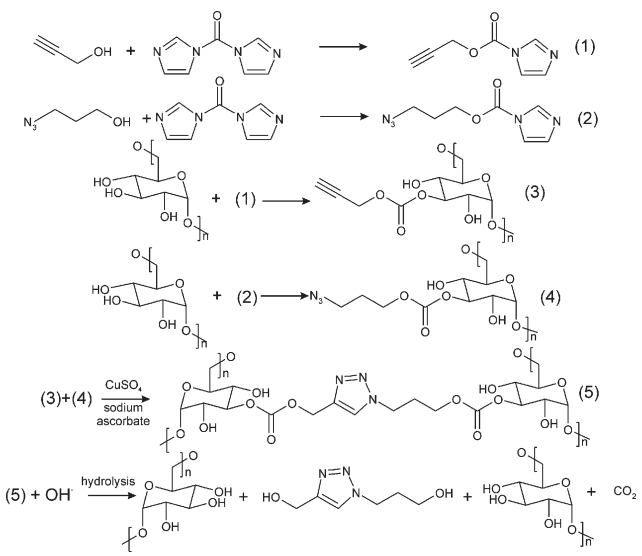
Dextrans modified with alkyne and azide groups through hydrolysable carbonate esters form degradable microcapsules after Cu^I catalysed 'click' reaction between azides and alkynes yielding triazole cross-links.

'Click' chemistry offers the possibility of carrying out covalent reactions with high selectivity and yield under extremely mild conditions.[1] The most widespread variant is the Cu^I-catalysed Huisgen reaction comprising the 1,3-dipolar cycloaddition of azides and alkynes forming a highly stable triazole compound.[1]

In this paper we apply 'click' chemistry for the preparation of microcapsules for drug delivery. Drug encapsulation in polymeric microparticles often requires the use of organic solvents or radical polymerization which may denaturize the therapeutic biomolecules (like peptides, proteins and nucleic acids) to be encapsulated.[2] The non-stringent reaction conditions, such as aqueous medium and ambient temperature, make 'click' chemistry highly attractive for the design of polymeric microcapsules. On the other hand, biodegradability is ubiquitous for the purpose of drug delivery.[2] We present a novel approach for the synthesis of biodegradable polymeric microcapsules based on the use of biodegradable 'click linkages'.

Dextrans (40 kDa) modified with respectively alkyne and azide groups which are connected to the dextran backbone through (biodegradable) hydrolysable carbonate esters (see Scheme 1) were cross-linked *via* 'click' chemistry. While others have reported on hydrogel formation *via* 'click' chemistry[3] or functionalisation of intrinsically degradable aliphatic polymers[4] by 'click' chemistry, we are to the best of our knowledge the first to introduce biodegradable 'click' linkages in macromolecular structures.

As shown in Scheme 1, dextran propargyl carbonate (dex-C≡C) and dextran azidopropyl carbonate (dex-N_3) are synthesized by activating propargyl alcohol and 3-azidopropanol, respectively with carbonyl diimidazole (CDI). Subsequently the activated compounds are grafted onto dextran chains resulting in the formation of a carbonate ester between the dextran backbone and the pending propargyl and azidopropyl moieties, respectively. Dex-C≡C and dex-N_3 with a degree of substitution (DS; *i.e.* the number of alkyne/azide moieties per 100 glucopyranose units) of respectively 20 (dex-C≡C and dex-N_3) and 5 (dex-N_3), as determined by ^{1}H-NMR spectroscopy, were synthesized. The covalent cross-linking of the dextran chains, through the formation of a triazole ring by 'clicking' dex-C≡C and dex-N_3, was performed in aqueous medium. An aqueous solution containing both dex-C≡C and dex-N_3 was emulsified in an external aqueous polyethylene glycol phase (note that dextran and polyethylene glycol do not mix at elevated concentrations).[5] The 'click' reaction was performed under standard conditions by addition of $CuSO_4$ and sodium ascorbate. After 30 min water was added and solid dextran hydrogel microcapsules were obtained after centrifugation and washing with pure water. The conversion of the azide and alkyne moieties to a triazole ring was demonstrated by attenuated reflection infrared spectrometry (ATR-IR). Spectra of the lyophilized dex-C≡C, dex-N_3 and 'clicked'microcapsules are shown in Fig. 1 and observed absorption bands are listed in Table 1. The characteristic azide peak is clearly visible at 2113 cm^{-1} in the case of dex-N_3 whereas this peak is significantly reduced in the spectrum of the 'clicked' microcapsules, indicating the consumption of the azide function.



Scheme 1 Reaction scheme of the synthesis of dextran propargyl carbonate (**3**) and dextran azidopropyl carbonate (**4**). Propargyl alcohol and 3-azidopropanol are activated with CDI yielding (**1**) and (**2**). Dextran is grafted with the activated compounds (**1**) and (**2**) yielding alkyne (**3**) and azide (**4**) modified dextran. Click reaction between (**3**) and (**4**) cross-links the dextran chains (**5**). Hydrolysis of the carbonate esters degrades the dextran network with the formation of dextran chains, CO_2 and a low molecular weight triazole compound as degradation products.

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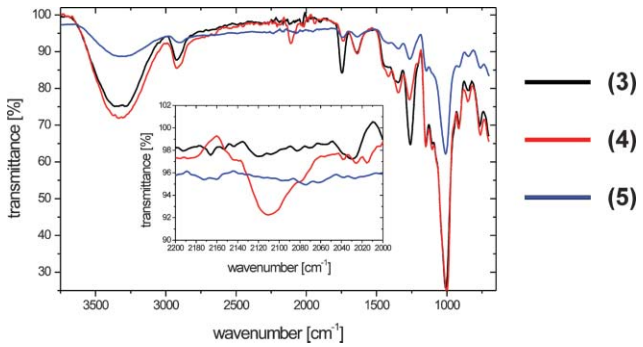

Fig. 1 ATR-IR spectra of the crude dex-C≡C (**3**), dex-N_3 (**4**) and 'clicked' microcapsules (**5**). The inset shows the region of the characteristic azide absorption band at 2113 cm^{-1}.

Table 1 IR-absorption bands for dex-C≡C, dex-N_3 and 'clicked' microcapsules

	dex-C≡C	dex-N_3	'clicked' microcapsules
950–1300 ($\nu_{C-O,\ ester}$)	detected	detected	detected
1450 (ν_{CH_3})	detected	detected	detected
1741 ($\nu_{C=O}$)	detected	detected	detected
2113 (ν_{N_3})	—	detected	—
2860–3000 (ν_{C-H})	detected	detected	detected
3296 ($\nu_{C-H,\ alkyne}$)	detected	—	—
3368 (broad, ν_{O-H})	detected	detected	detected

Confocal microscopy (Fig. 2A) shows the obtained microcapsules while scanning electron microscopy (Fig. 2B) depicts the obtained three-dimensional structure of the microcapsules, confirming the formation of solid microcapsules upon 'clicking' of the dex-C≡C and dex-N_3 chains. To show the potential of the proposed strategy for the design of drug delivery systems, fluorescein isothiocyanate dextran (FITC-dextran; 150 kDa) was encapsulated as a model drug (inset of Fig. 2A).

Each cross-link in the hydrogel network, which arises from the triazole ring formation, is connected to the dextran through two carbonate esters. It is known that such carbonate esters hydrolyse under physiological conditions. The reaction of the alkaline hydrolysis of the hydrogels is shown in Scheme 1. Upon cleavage of the carbonate esters, the original dextrans (as used for the synthesis of dex-N_3 and dex-C≡C) are obtained as degradation products along with CO_2 and a low molecular weight triazole compound. To show the proof of principle of microcapsule degradation we accelerated the degradation rate by increasing the pH and the microcapsule behavior was monitored by confocal microscopy (Fig. 2 C and D). It is known that at alkaline pH the degradation of the carbonate esters occurs within seconds, compared to days under physiological conditions.[6] As Figs. 2 C and D show, the microcapsules gradually swell and dissolve in the surrounding solution upon addition of a drop of 1 M NaOH, releasing the encapsulated content, thus demonstrating the (bio)degradability of the 'clicked' microcapsules.

In the next step we aimed to study the degradation of the 'clicked' microcapsules under physiological conditions (*i.e.* pH 7.4, 0.15 M NaCl and 37 °C). Therefore FITC-dextran (2000 kDa; 10% (w/w)) was encapsulated in 'clicked' microcapsules having respectively a higher (by combination of dex-C≡C DS 20 and dex-N_3 DS 20) and lower (by combination of dex-C≡C DS 20 and dex-N_3 DS 5) network density. The microcapsules were incubated under physiological conditions and samples were withdrawn at regular time intervals until no microcapsules remained in the test tube (as checked by optical microscopy).

The cumulative release curves in Fig. 2E show an initial burst release (due to passive diffusion of weakly entrapped FITC-dextran), followed by a sustained (degradation controlled) release. Secondly, 'clicked' microcapsules with a higher network density (dex-C≡C$^{(DS\ 20)}$/dex-$N_3^{(DS\ 20)}$) exhibit a lower burst release (9%) than those with a lower network density (20% for dex-C≡C$^{(DS\ 20)}$/dex-$N_3^{(DS\ 5)}$). Further, clicked microcapsules with a higher network density exhibit a slower sustained release (leveling of 20 days for dex-C≡C$^{(DS\ 20)}$/dex-$N_3^{(DS\ 20)}$ *vs.* 10 days for dex-C≡C$^{(DS\ 20)}$/dex-$N_3^{(DS\ 5)}$) which could be expected as more cross-links have to be hydrolysed to sufficiently enlarge the mesh size in the network, required to release the entrapped macromolecules.

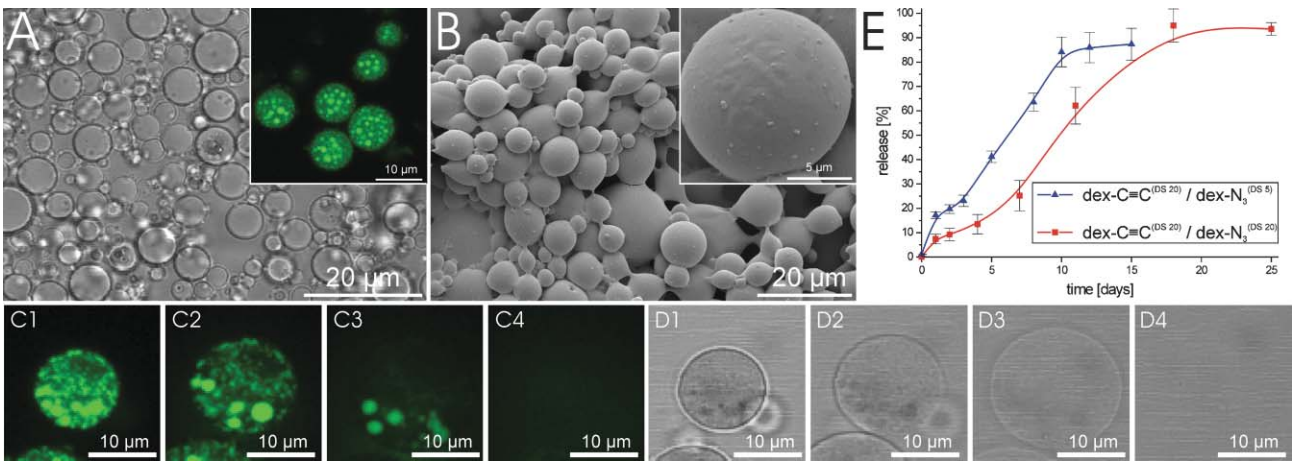

Fig. 2 Confocal transmission (A) and scanning electron (B) microscopy images of microcapsules obtained by 'clicking' dex-N_3 and dex-C≡C. Confocal fluorescence (C1–C4) and transmission (D1–D4) microscopy snapshots of degrading 'clicked' microcapsules (the time interval is 2 s). The inset in pane (A) shows the fluorescence image of FITC-dextran encapsulated in 'clicked' microcapsules. (E) Release profiles of FITC-dextran (2000 kDa) from 'clicked' microcapsules under physiological conditions (the values are the main values of two independent measurements).

In conclusion we have shown that biodegradable dextran hydrogel microcapsules can be synthesized *via* 'click' chemistry. It was demonstrated that the 'clicked' microcapsules can encapsulate macromolecular compounds and release them in a tailored, controlled fashion. The mild, non-harmful reaction conditions for 'click' chemistry are especially attractive when labile biological drugs have to be encapsulated. Therefore it is believed that biodegradable 'click' chemistry will become an important strategy for the design of micro- and nanosized drug delivery systems.

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