pestle in a mortar for 30 min. Nanoparticles were then prepared using an amount of mixture containing 100 mg CyA.

NMR spectra of a solution of the CyA/HPβCD mixture in CD_3OD (99.8%D) were recorded at 30 °C on a Bruker DRX 400 NMR spectrometer operating at 400 MHz. 64 scans were accumulated.

In order to study the release of CyA, 40 mg of freeze-dried nanoparticles were accurately weighed and transferred to a glass vial containing 10 ml PBS pH 7.4. The vials were oscillated in a water bath at 32 °C. To avoid water evaporation, the vials were covered with rubber caps. Throughout the experiment 4 ml sample was withdrawn at specified time intervals and replaced by an equal volume of fresh buffer solution. The sample was centrifuged for 3 h (4000 rpm, 32 °C) and the CyA concentration of the supernatant was determined by a validated HPLC method.

Result and discussion

In a CyA/HPβCD inclusion complex it is expected that the lipophilic side chains of CyA will enter the hydrophobic cavity of HPβCD and that shielding of methyl groups will be observed. Therefore the aliphatic region (0.7–1.7 ppm) of the NMR spectrum of the CyA/HβPCD sample was compared in detail to the spectrum of a CyA solution [5]. No substantial differences (<0.003 ppm) could be detected hence there is no evidence for complexation. Considering the large structure of CyA containing 8 lipophilic side chains, a possible explanation may be that a dynamic equilibrium, whereby all different lipophilic side chains only enter the hydrophobic cavity for a relatively short time (i.e. on the average 1/8 of the total time), leads to a proportional reduction of the shielding effect.

The cumulative amount of CyA released from the nanoparticles was determined. The results are reported as mean ± SD of three experiments in Table 1. In Fig. 1 the release curves are presented. An initial burst effect followed by a slower release rate was observed. At each time interval, the amount of CyA released is about 5% higher in the presence of HPβCD resulting in a cumulative release of 63% and 81% after 27 h from nanoparticles loaded by respectively CyA and CyA/HPβCD mixture.

Table 1
The cumulative percentage of CyA release from CyA and CyA/HPβCD loaded nanoparticles.

Time (min)	CyA release (%) ± SD n = 3	
	CyA loaded nanoparticles	CyA/HPβCD loaded nanoparticles
240	37 ± 1	42 ± 3
360	51 ± 1	61 ± 1
540	60 ± 0	71 ± 2
1620	63 ± 1	81 ± 2

Taking into account the NMR results, the improvement of CyA release in the presence of HPβCD should not be due to formation of a complex with higher aqueous solubility, but to the formation of pores inside the PLGA nanoparticles.

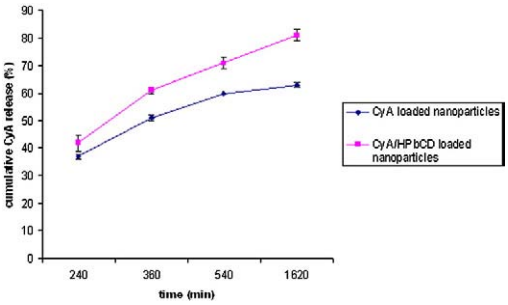

Fig. 1. Release curves of CyA from PLGA nanoparticles (mean ± SD, n = 3).

Conclusion

NMR spectroscopy of the prepared CyA/HPβCD mixture showed no evidence for complexation of CyA with HPβCD. Nevertheless, an improvement of CyA release from PLGA nanoparticles containing the CyA/HPβCD mixture compared to CyA loaded nanoparticles is obtained.

Acknowledgement

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Rapid gelation of injectable hydrogels based on hyaluronic acid and poly(ethylene glycol) via Michael-type addition

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Abstract summary

Injectable hydrogels were formed by a Michael-type addition reaction between thiolated poly(ethylene glycol) (PEG) and maleimide-functionalized hyaluronic acid (HA). Depending on the polymer concentration and the degree of substitution (DS) of HA with maleimide groups, the gelation time and storage moduli of the hydrogels can be varied. The gelation time ranged from less than 5 s to about 0.5 min and storage moduli ranged from 39 to 375 Pa. Moreover, frequency and strain sweeps demonstrated the elastic nature of these hydrogels.

Introduction

Injectable hydrogels are generally formed by mixing two precursor solutions that gelate *in situ* by crosslinking. They are attractive for biomedical applications because a minimally invasive procedure is employed and cells and bioactive molecules can be easily and homogeneously incorporated prior to gelation. Michael-type addition is a commonly used crosslinking method to obtain injectable hydrogels. In this study, maleimide-functionalized hyaluronic acids (HA-Mal) with different degree of substitution (DS) were crosslinked with thiolated 4-arm poly(ethylene glycol) (PEG-4SH, MW = 10 kg/mol) via Michael-type addition. The influences of the DS and polymer concentration on the gelation time and modulus were studied.

Experimental methods

Polymer synthesis

Hyaluronic acid (HA) (MW = 45 kg/mol, measured by viscosity) was functionalized with maleimide moieties according to previously

reported method [1]. Briefly, HA (100 mg) was dissolved in 50 mL of 0.1 M MES buffer. To the solution, EDC (144 mg, 0.75 mmol) and NHS (86 mg, 0.75 mmol) were added and the resulting mixture was stirred for 30 min. Afterwards, N-(2-aminoethyl) maleimide trifluoroacetate salt (AEM) (63.5 mg, 0.25 mmol) was dissolved in 50 mL of 0.1 M MES buffer and added to the mixture. After 4 h, the mixture was dialyzed first against 50 mM NaCl and then deionized water. The polymers were obtained as a white foam after freeze-drying. The DS, defined as the number of maleimide groups per 100 disaccharide rings of HA, was determined by ^{1}H NMR.

Hydrogel preparation and gelation time

Hydrogel samples (~0.25 mL) were prepared in vials by mixing PBS solutions of HA-Mal and PEG-4SH. The polymer concentration (1–3% w/v) was defined as the percentage of total polymer weight per volume of PBS solution. A thiol/maleimide molar ratio of 1.2 was applied in the preparation of the hydrogels. The gelation time was determined by the vial tilting method.

Rheological analysis

Rheological experiments were carried out with a MCR 301 rheometer (Anton Paar) using a parallel plate (25 mm diameter, 0°) configuration at 37 °C in oscillatory mode. A layer of oil was introduced around the sample to avoid water evaporation. The evolution of the storage (G′) and loss (G″) modulus was recorded as a function of time at a frequency of 1 Hz and a strain of 1%. After the oscillation experiment, a frequency sweep was performed with the frequency varying from 0.01 to 10 Hz and at 1% applied strain. Finally, a strain sweep was performed varying from 0.1% to 10% at a frequency of 1 Hz.

Result and discussion

Polymer synthesis

Maleimide-functionalized HA (HA-Mal) polymers were prepared by reacting HA with AEM using EDC/NHS activation (Fig. 1). The reaction was performed in 0.1 MES buffer solutions with different molar feed ratios of carboxylic acid groups of HA to amino groups of AEM. After 4 h reaction, the resulting HA-Mal polymers were purified by dialysis and isolated by freeze-drying. The ^{1}H NMR spectrum of HA-Mal showed that a signal at δ 7.0 was present corresponding to the vinyl protons of the maleimide groups (Mal), compared to the spectrum of unmodified HA (Fig. 2). The degree of substitution (DS) of HA-Mal, defined as the number of maleimide groups per 100 disaccharide rings of HA, was determined from the ^{1}H NMR spectra by comparing the integrals of signals at δ 2.0 and 7.0, attributed to the acetamide methyl protons in HA and vinyl protons of the maleimide moieties, respectively. The DS of HA-Mal increased from 4 to 7 when the [NH_2]/[COOH] feed ratio was increased from 1 to 2.

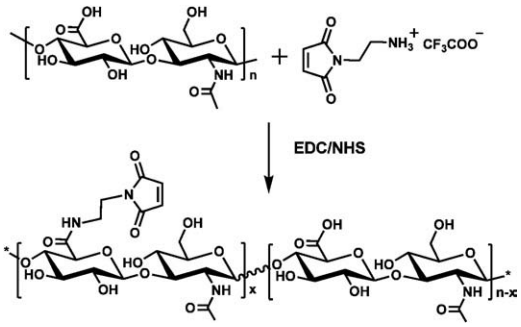

Fig. 1. Synthesis of maleimide-functionalized HA (HA-Mal) using EDC/NHS activation.

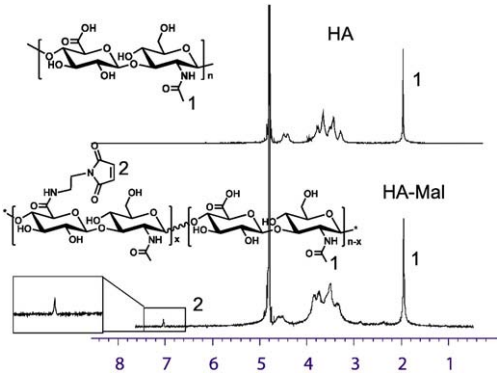

Fig. 2. ^{1}H NMR spectra of HA and HA-Mal (D_2O).

Hydrogel formation

Mixing a solution of HA-Mal in PBS with a solution of PEG-4SH in PBS induced rapid hydrogel formation. In Fig. 3 it is shown that an increase in the polymer concentration from 1 to 3% (w/v) results in a decrease in gelation time. For example, for the hydrogels prepared from HA-Mal with a DS of 4, the gelation time is about 35 s for the 1% (w/v) hydrogels, but only 10 s for the 3% (w/v) hydrogels. Moreover, hydrogels based on HA-Mal with a DS of 7 had shorter gelation times than those with a DS of 4.

In general, the gelation times for HA-Mal/PEG-4SH hydrogels ranged from less than 5 s to about 0.5 min. Obviously, the high reactivity between maleimide and thiol groups makes this in-situ hydrogel formation highly interesting for application in tissue regeneration and as drug delivery systems.

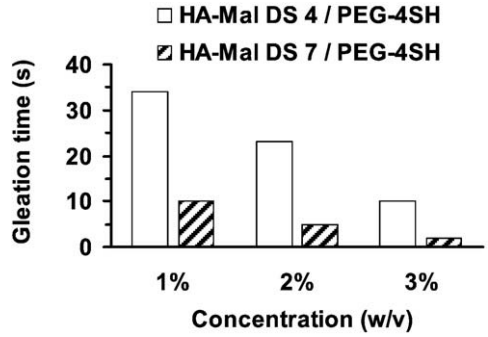

Fig. 3. Gelation time of hydrogels prepared from PEG-4SH and HA-Mal with a DS of 4 and 7 as a function of total polymer concentration.

Rheological analysis

Rheological analysis was used to determine the storage modulus, loss modulus and damping factor of the HA-Mal/PEG-4SH hydrogels as a function of time (Fig. 4). In general, the storage moduli increased when the polymer concentration was increased from 1 to 3% (w/v) most likely due to the increased crosslinking density. Hydrogels from HA-Mal with a DS of 7 showed a higher G′ compared to hydrogels based on HA-Mal with a DS of 4. The hydrogels from PEG-4SH crosslinked with HA-Mal DS 7 at a polymer concentration of 3% (w/v) showed the highest storage modulus of 375 Pa. Besides, the damping factor of the hydrogels prepared from HA-Mal DS of 7 ranged from 0.01 to 0.04, which is much lower than those prepared from HA DS of 4 (0.1–0.3). This indicated that the hydrogels with a higher DS were more elastic.

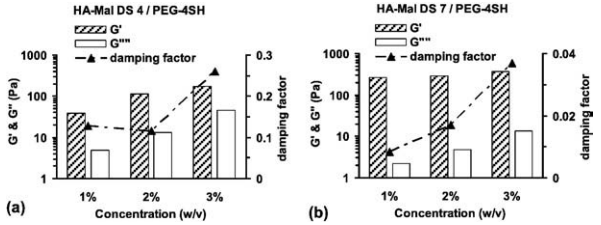

Fig. 4. Storage modulus (G′), loss modulus (G″) and damping factor of the hydrogels prepared from PEG-4SH and HA-Mal with a DS of (a) 4 or (b) 7 as a function of time.

Frequency and strain sweeps of HA-Mal/PEG-4SH hydrogels are presented in Fig. 5. A frequency sweep conducted at a strain of 1% revealed that G′ was relatively independent of frequency within the frequency range of 0.01−10 Hz for all the hydrogels, confirming that these materials behaved as an elastic solid. For all the hydrogels, strain sweep experiments (0.1−10%) were performed at an oscillatory frequency of 1 Hz. It shows that a strain ranging from 0.1% to 10% is in the linear viscoelastic range for all tested hydrogels.

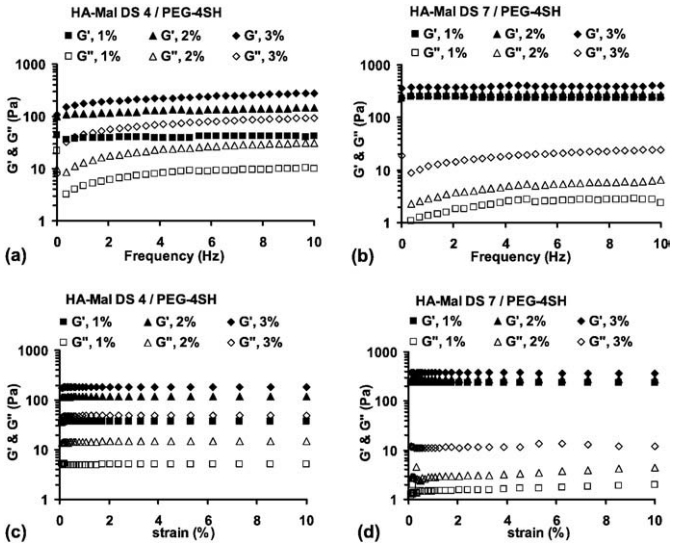

Fig. 5. Frequency and strain sweep of hydrogels prepared from PEG-4SH and HA-Mal with a DS of 4 or 7 as a function of (a and b) frequency and (c and d) strain.

Conclusion

We demonstrated that injectable hydrogels from thiolated PEG and Maleimide-functionalized HA can be prepared by Michael-type addition. The hydrogels showed fast gelation ranging from less than 5 s to about 0.5 min. Besides, storage moduli of the hydrogels can be easily adjusted by the polymer concentration and DS of maleimide moieties. Rheological analysis demonstrated that these gels were elastic.

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Mesoporous silicon microparticles as carriers for peptides

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Abstract summary

Porous silicon is considered to be an interesting and safe material for drug delivery. Mesoporous thermally hydrocarbonized silicon (THCPSi) microparticles were examined as peptide carrier material for a model peptide, melanotan II (MTII). THCPSi microparticles showed to sustain the MTII the release in vitro. Moreover, THCPSi microparticles changed the profile of pharmacological response curves of MTII in vivo, indicating sustained release. In conclusion, THCPSi microparticles are promising carrier material for sustained peptide release.

Introduction

Peptides are interesting molecules for drug development, because they could serve as better medical therapies in various pathological conditions. However, peptide delivery interfaces many challenges, such as low oral bioavailability, which typically lead on to use of parenteral administration routes. In addition, the biological half-life of peptides is very short, which generates a need to develop controlled release systems in order to prolong the duration of action and to lower the frequency of administration.

Particulate drug delivery systems are one possibility to enhance peptide delivery. Porous silicon (PSi) offers a number of advantageous properties as a drug carrier material [1], such as a high adsorbing surface area and easily controllable pore size. In the present study THCPSi microparticles were investigated in peptide delivery in vitro and in vivo. Melanotan II (MTII), of which pharmacological actions include induction of heart rate and inhibition of water consumption [2,3], was used as model a peptide.

Experimental methods

The unloaded microparticles (38–53 μm) were prepared as described previously [4]. The present peptide was loaded into microparticles from methanol solution in two batches (the loading degrees were 14.8–15.1 w/w %). In vitro release was examined in sink conditions in pH 7.4 buffer (+37 °C) and MTII was detected by HPLC. Male Wistar rats and CD_2F_1 (Balb/c x DBA2) hybrid male mice were purchased from National Laboratory Animal Center (Kuopio, Finland) at the age of 7–8 weeks. They were housed individually in a regulated environment; temperature 22 ± 1 °C, relative air humidity 55 ± 15% and 12/12 h light/dark cycle with lights on at 7 am. Commercial pellets (Lactamin R36, Sweden) and tap water were available ad libitum, unless otherwise indicated.

The Institutional Animal Care and Use Committee of the Provincial Government approved the experiments. Procedures were conducted in accordance with the guidelines set by the European Community Council Directives 86/609/EEC.

For the *in vivo* experiments, the animals were divided into following treatment groups: 1) 0.5% carboxymethylcellulose sodium in 0.9% NaCl (Vehicle), 2) unloaded THCPSi microparticles suspended in the vehicle (THCPSi), 3) peptide loaded THCPSi microparticles suspended in vehicle (THCPSi+MTII) and 4) the peptide dissolved in vehicle (MTII).

Water consumption measurements

Male mice (Balb/c x DBA2) were acclimatized to conditions similar to the experiment and fasted 16 h before the measurements. All the treatments were injected subcutaneously with a 3.7 mg/kg MTII dose.