Increased \textit{in-vitro} release time of poorly soluble drugs loaded into injectable crosslinked hyaluronic acid hydrogel formulations

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\textbf{ABSTRACT SUMMARY}

Hyaluronic acid from \textit{Bacillus subtilis} was crosslinked with divinyl sulfone to produce hydrogels for controlled release formulations of a range of poorly soluble drugs. Release studies showed a significant increase in release time when the drugs were loaded into the cross-linked hyaluronic acid hydrogel formulations compared with reference API suspensions.

\textbf{INTRODUCTION}

Hyaluronic acid (HA) is a natural linear and unbranched polysaccharide belonging to the class of non-sulphated glycosaminoglycans. HA is composed of beta-1,3-N-acetyl glucosamine and beta-1,4-glucuronic acid repeating disaccharide units (Figure 1).

\begin{figure}[ht]
  \centering
  \includegraphics[width=0.5\textwidth]{figure1.png}
  \caption{Chemical structure of the repeating disaccharide unit in HA.}
\end{figure}

HA is ubiquitous in human and animal tissues, where it exhibits significant structural, rheological, physiological, and biological functions that make it an attractive carrier for drug delivery applications$^1$. HA is recognized as a high-value biopolymer with numerous proven and marketed applications within the cosmetic, biomedical and pharmaceutical fields. However, HA injected in the body is typically degraded rapidly limiting the long term effects of the polymer. To overcome the short residence time several modification technologies have been investigated. In this work HA produced by fermentation of \textit{Bacillus subtilis} strain has been crosslinked with divinyl sulfone (DVS) to produce hydrogels suitable for pharmaceutical applications (Figure 2). Several poorly soluble small molecule drugs were used as models and loaded into the crosslinked HA hydrogels yielding depot sustained release formulations. The drugs used in this work were carbamazepine (antiepileptic), triamcinolone acetonide (anti-inflammatory steroid), triamcinolone hexacetonide (anti-inflammatory steroid), diclofenac (non-steroid anti-inflammatory drug NSAID) and odanacatib (treatment for osteoporosis and bone metastasis). The drugs were chosen to mimic formulations for both intra-articular (IA) injection in treatment of osteoarthritis and subcutaneous depot (SC) within a range of treatments.

\textbf{EXPERIMENTAL METHODS}

The HA was produced by Novozymes Biopharma DK A/S by fermentation of \textit{Bacillus subtilis}. The average molecular weight of the starting materials was 0.7-1.0 MDa.

\begin{figure}[ht]
  \centering
  \includegraphics[width=0.5\textwidth]{figure2.png}
  \caption{Reaction of primary alcohols in HA and divinyl sulfone resulting in crosslinked HA hydrogels.}
\end{figure}

The hydrogels were prepared with HA/DVS weight ratios in the range of 5:1 to 30:1 resulting in hydrogels with a final HA concentration of 1.9 to 1.2% w/w according to the method described.$^2$ The crosslinked HA hydrogels were neutralized and swollen in PBS buffer yielding hydrogels with a pH of 6.8-7.2. Finally the hydrogels were micronized into particles of 200-500 \mu m using a fine metal mesh and mixed with 10-20% w/w of a HA solution of 20-50 mg/mL.

The API’s were loaded into the hydrogel particles by mixing dry API powder into the formulation. The final drug concentrations are listed in Table 2. The reference formulations were mixtures of API powder suspended in PBS buffer solution and propylene glycol to keep the API well suspended during storage.

The drug release from the crosslinked HA hydrogel formulations were assessed by a dissolution method using a closed loop system configuration (SOTAX CE7smart) and the USP 4 dissolution method with 22.4 mm test cells. Dialysis membrane inserts equipped with MWCO membranes with pore sizes of 1 MDa were used. 500-1000 mL PBS buffer with pH 6.9 was used as medium equilibrated at 37 °C. The flow rate was set to 4 mL/min and a high stirring speed was used. The drugs were detected on-line with UV absorbance at 230-275 nm depending on the API. The tested API dose and the volume injected into the dissolution system are listed in Table 1.
RESULTS AND DISCUSSION

Crosslinked HA hydrogels were prepared and formulated with the APIs resulting in a white, drug loaded hydrogel formulation. The release of the tested drugs from the hydrogel formulations were followed by UV detection with short measuring intervals (minutes and hours).

Release profiles for all the reference drug suspensions are given in Figure 3. For the reference formulations complete drug release were achieved after 6 hours for triamcinolone acetonide and up to 2.2 days for carbamazepine. The release profiles of all the tested drugs from crosslinked HA hydrogels are given in Figure 4 and the complete drug release time (95%) are listed in Table 2. The API showing the largest difference in release time is triamcinolone acetonide where the complete release was extended from 6 hours up to 58 days.

Table 2. Release time and composition of crosslinked HA hydrogel formulations.

<table>
<thead>
<tr>
<th>API in formulation (Abbreviation)</th>
<th>API conc. (mg/g)*</th>
<th>Hydrogel crosslinking degree (HA/DVS)</th>
<th>Release time at 95% (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine (CM)</td>
<td>50</td>
<td>30:1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20:1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10:1</td>
<td>21</td>
</tr>
<tr>
<td>Triamcinolone acetonide (TA)</td>
<td>30</td>
<td>10:1</td>
<td>&gt;58</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10:1</td>
<td>2</td>
</tr>
<tr>
<td>Triamcinolone hexacetonide (THA)</td>
<td>10</td>
<td>5:1</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Diclofenac (DF)</td>
<td>60</td>
<td>10:1</td>
<td>13</td>
</tr>
<tr>
<td>Odanacatib (ODA)</td>
<td>40</td>
<td>5:1</td>
<td>30</td>
</tr>
</tbody>
</table>

*All tested formulations where compared to a reference suspension of the API with the same concentration.

CONCLUSION

In this work, it has been shown that in-vitro release of a range of poorly soluble drugs can be sustained when loaded into crosslinked HA hydrogel formulations. The release was significantly prolonged compared to a reference drug/API suspension. This can potentially lead to formulation of poorly soluble drugs in high doses to be released in-vivo over a period of up to two months, resulting in a decrease in number of administrations and increased patient compliance.

REFERENCES