Diffusion of fluorescent insulin from an acrylic derivatised dextran-concanavalin A gel in an implantable closed loop insulin delivery device

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ABSTRACT SUMMARY
The drug delivery mechanism of fluorescently labelled insulin (FITC-Insulin) from a glucose sensitive gel held in an implantable closed loop insulin delivery device was found to be diffusion controlled. The diffusion coefficient of FITC-Insulin in the gel was determined and response to glucose triggers assessed.

INTRODUCTION
A fully functional implantable insulin delivery device which has shown to control blood glucose in diabetic rats and more recently in diabetic pigs has been developed [1, 2]. The device encases a reversible glucose-sensitive gel of co-polymerised dextran methacrylate (dex-MA) and concanavalin A methacrylamide (conA-MA), which produces a reversible change in consistency on contact with glucose to modulate insulin transport and thus acts as a self adjusting system. The device, implanted in the peritoneal cavity, works on a very fast feedback mechanism for controlling insulin release, mimicking a normal pancreas enabling to overcome inherent problems encountered in maintaining normoglycemia with current electronically or biologically based closed loop systems. In this work we present the drug release kinetics data for FITC-Insulin from the gel used in the device.

EXPERIMENTAL METHODS

Synthesis and characterisation of fluorescently labelled insulin
Human Insulin was labelled with fluorescein isothiocyanate (FITC). Briefly, 10mg/ml human insulin in 0.1M phosphate buffer containing 200µM EDTA (pH 7.0) was prepared. FITC solution prepared in acetone (5mg/mL) was added dropwise to the human insulin stock solution (3:1 molar ratio) at pH 7.0 in the dark and stirred for 25 hours. After this time the reaction was stopped and the FITC-Insulin conjugates were fractionated by Gel Permeation Chromatography using a Sephadex G25 column. Fractionated FITC-Insulin conjugates were characterised using HPLC and El Mass Spectroscopy [4]. The FITC-Insulin synthesised were predominantly di-labelled conjugates and contained no unlabelled insulin.

Preparation of gel
The preparation of the gel has been described previously [3]. Briefly, Con-MA (100mg) was dissolved in distilled water and photoinitiator Irgacure® (0.178 µmoles) was added. Dex-MA (RMM 500Da) (100mg) was added to the mixture and stirred to form a viscous solution, covered in foil and allowed to stand for 2 hours; the mixture was then placed between two glass plates separated by a 60µm thick gasket and was irradiated under UV-light (365nm, 10mJ cm⁻²) for 5 minutes. The crosslinked gel (CLgel) formed was stored aseptically at 4°C for at least 24 hours prior to use. A non-polymerised version of the gel (NPgel) containing derivatised conA and dextran (RMM 2000kDa) was prepared in the same way but not irradiated.

Evaluation of drug delivery mechanism
An experimental set-up was designed using the same InSmart device as was used in in vivo pig trials previously [2] as shown in Figure 1.

![Figure 1: Schematic of the experimental set-up.](image)

FITC-Insulin diffusing through the gel from the donor side to the receptor side was monitored over time by conversion of fluorescence intensity recordings at 518nm into concentration values using calibration curves. Preliminary experiments using probe dye fluorescein sodium and using NPgel aided selection of appropriate mathematical models.

The Power Law (Peppas equation): The release of fluorescein sodium from the NPgel was recorded over time and applied to the power law shown below to determine if the drug delivery mechanism was Fickian diffusion.

\[
\frac{M_t}{M_{\infty}} = kt^n
\]

Where, \(M_t\) and \(M_{\infty}\) are the absolute cumulative amount of drug released at time \(t\) and infinite time, respectively; \(k\) is
a constant incorporating structural and geometric characteristics of the system and \( n \) is the diffusional exponent, which is indicative of the mechanism of drug release.

**Mathematical models for determination of Diffusion coefficient:** The diffusion cell technique – Quasi steady state (QSS), the Time lag (TL) slope and the TL intercept methods based on Fick’s law were identified for determination of diffusion coefficient of FITC-Insulin [5].

**QSS:**

\[
\ln \frac{C_{L_A(t)}}{C_{L_B(t)}} - \ln \frac{C_{L_A(0)}}{C_{L_B(0)}} = -\frac{D_e}{V} A_g \left( \frac{1}{V_A} + \frac{1}{V_B} \right) (t - t_0)
\]

\( D_e \) is calculated from the slope of the line obtained by plotting the left-hand side of equation against time \( t \).

**TL slope:**

\[
Q_t = V_b C_{L_B(t)} = \frac{A_g D C_{L_A(0)}}{l} \left( t - \frac{l^2}{6D} \right)
\]

\( D \) is calculated from the total amount of solute transferred through the gel \( (Q_t) \) against time \( (t) \).

**TL intercept:**

\[
t_{lag} = \frac{l^2}{6D}
\]

\( D \) is calculated from the lag time, \( t_{lag} \), the intercept of the linear part of the TL plot.

(Key: \( D_e \) & \( D \) are the effective diffusion coefficient and diffusion coefficient, \( V_A \) & \( V_B \) volumes of the two chambers separated by the gel, \( A_g \) is effective membrane area, \( l \) is gel thickness, \( C_{L_A} \) & \( C_{L_B} \) are the concentration of the drug solute in the two chambers at initial time \( t_0 \) or at time \( t \).

**Glucose sensitivity** of the gel was assessed by challenging the system with increasing physiologically relevant concentrations of glucose (0.1% to 1%).

**RESULTS AND DISCUSSION**

**The Power Law:** The gel (thickness 1.17 mm) housed in the device can be considered as a thin film or slab with no edge effects and the power law fit for the release of fluorescein sodium through the gel indicates diffusion controlled release (Figure 2).

**Determination of Diffusion coefficient:** Table 1 shows diffusion coefficients from mathematical models for FITC-Insulin in NPgel and CLgel with and without 0.1% basal glucose.

<table>
<thead>
<tr>
<th>Gel</th>
<th>QSS method ( D_e (m^2/s) )</th>
<th>TL Slope method ( D (m^2/s) )</th>
<th>TL Int. method ( D (m^2/s) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPgel</td>
<td>1.05E-11</td>
<td>1.04E-11</td>
<td>1.12E-11</td>
</tr>
<tr>
<td>NPgel + 0.1% Glucose</td>
<td>4.52E-12</td>
<td>4.58E-12</td>
<td>2.24E-11</td>
</tr>
<tr>
<td>CLgel</td>
<td>7.54E-12</td>
<td>7.52E-12</td>
<td>1.44E-11</td>
</tr>
<tr>
<td>CLgel + 0.1% Glucose</td>
<td>5.90E-12</td>
<td>5.89E-12</td>
<td>9.92E-11</td>
</tr>
</tbody>
</table>

Table 1: Diffusion coefficient values (\( D_e \) and \( D \)) for FITC-Insulin in NPgel and CLgel with and without 0.1% basal glucose determined using the QSS method, the TL slope method and the TL intercept method.

The QSS and TL methods gave reliable correlation in diffusion coefficient values for FITC Insulin in NPgel and CLgel. The TL intercept method gave different values, approximately a decade faster in some instances, which is consistent with the findings of others [5].

**Glucose sensitivity:** Figure 3 shows increasing \( D \) values with increasing glucose concentrations in NPgel.

**Figure 3:** FITC-Insulin diffusion coefficient values with increasing glucose concentration in NPgel.

**CONCLUSION**

The drug release mechanism from the glucose sensitive gel held in an implantable closed loop insulin delivery device was found to be diffusion controlled. The diffusion coefficient for FITC-Insulin in the NPgel was 1.05E-11 m²/s and in the CLgel was 7.53E-12 m²/s. The \( D \) values increase with increasing glucose concentrations, device thus showing glucose sensitivity, mimicking pancreas.

**REFERENCES**


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