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Chemical Papers 65 (6) 903–908 (2011)
DOI: 10.2478/s11696-011-0059-5

ORIGINAL PAPER

Methylprednisolone release from agar–Carbomer-based hydrogel: a promising tool for local drug delivery

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Received 14 March 2011; Revised 12 May 2011; Accepted 19 May 2011

A number of studies and works in drug delivery literature are focused on the understanding and modelling of transport phenomena, the pivotal point of a good scaffold design for tissue engineering. Accurate knowledge of the diffusion coefficient of an active drug plays a key role in the analysis, prediction of their kinetics and formulation of efficient drug delivery systems. In this work, the kinetics of the release of methylprednisolone from agar–Carbomer hydrogel were studied taking into consideration the different drug concentrations and clearances typically achieved in in vitro or in vivo tests. Starting from the experiments it is possible to model the transport phenomenon and to calculate the diffusion coefficient through the hydrogel matrix.

Keywords: biomaterials, diffusion, drug delivery, hydrogel, methylprednisolone

Introduction

Tissue engineering, which may be briefly characterised as the smart combination of cells and materials to replace damaged or missing parts of living tissues, is widely accepted as representing the future in regenerative medicine and healthcare (Badylak & Nerem, 2010; Langer & Vacanti, 1993; Lanza et al., 2000; Perale et al., 2011b; Sakurada et al., 2008). New highlights suggest the combined use of cells and materials together with specific and appropriate drugs (Katz & Burdick, 2009; Kim et al., 2009; Sakurada et al., 2008). Out of all the known and proposed materials (Mouriño & Boccaccini, 2010; Shoichet, 2010; Slaughter et al., 2009), the emerging strategies in regenerative medicine confirm a very strong interest in hydrogel as a major candidate for both cell and drug delivery (Arosio et al., 2010; Katz & Burdick, 2009; Kim et al., 2009; Slaughter et al., 2009), allowing the building of water-based systems with cells directly included inside the gel and drugs dissolved within them (Loh et al., 2010; Tang et al., 2010). This permits the incorporation of both specific drugs for cell-supporting and also active molecules for local delivery into the target tissue (Katz & Burdick, 2009; Santoro et al., 2011; Slaughter et al., 2009).

Thus the investigation of drug release mechanisms in these matrices plays a key role in the design of smart systems for tissue engineering (Lin & Metters, 2006; Perale et al., 2009). Even though if the literature widely provides both experimental studies and phenomenological theories relating to the diffusion mechanism of small molecules from macromolecular networks, such as polymeric hydrogels, the available data often show low or even no accordance with the descriptive theories and, in general, this framework is still highly controversial (Alexis, 2005; Johansson et al., 1991; Sant et al., 2008). Moreover, deep understanding of the phenomena involved and the physical and chemical comprehensible models are sometimes missing.

Within this framework, a controlled drug delivery from a new hydrogel formulation, intended for regenerative medicine (Perale et al., 2011a, 2011c), was investigated, focusing on gaining knowledge of the transport phenomena from these gels. Investiga-

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tions were performed using biodegradable polymeric hydrogel loaded with methylprednisolone (MP). This hydrogel type was selected due to its promising behaviour as a cell carrier in spinal cord injury repair (Perale et al., 2011a, 2011c). The choice of methylprednisolone, a synthetic glucocorticoid drug, follows the same rationale, as it is the active compound most frequently employed in the pharmacological treatment of acute spinal cord injury (Bracken et al., 1990; Cao et al., 2010; Kim et al., 2009). Drug release experiments were carried out in order to identify the key parameters which influence transport phenomena and to estimate the MP diffusion coefficient (Falk et al., 2004).

Experimental

The hydrogel selected is synthesised from two polymers, respectively: Carbomer 974P (CAS 151687-96-6, Fagron, the Netherlands) and agarose (CAS 9012-36-6, Invitrogen Corp., USA), both of 0.25 mass % and both being pharmaceutical grade solubilised in phosphate-buffered saline solution (PBS) (Rossi et al., 2010). Propylene glycol (CAS 504-63-2, Sigma, Germany) and glycerol (CAS 56-81-5, Merck Chemicals, Germany) were added as cross-linking agents of 30 mass % and 0.5 mass %, respectively; triethylamine (CAS 121-44-8, Sigma, Germany) was added also in order to maintain the solution at neutral pH. The suspension was agitated and then heated electromagnetically to 80 °C to induce condensation reactions. During cooling, while the mixture was still liquid and stirred, MP (Kim et al., 2009) ($M_w = 374.48$ Da, CAS 83-43-2, solubility in water 78 μg mL^{-1}) (Stella et al., 1995) in the form of ultra-micronised pure powder, MicroSphere S/A (Switzerland)), was loaded into hydrogel at three different concentrations: 28.6 μg mL^{-1} (designated as AC-MP_H), 9.5 μg mL^{-1} (designated as AC-MP_M), and 0.57 μg mL^{-1} (designated as AC-MP_L), respectively. The gelation took place in a 48 multi-well plate: the samples obtained were cylindrical with a volume of 0.5 mL each (diameter $d = 1.1$ cm), thus reasonably resembling the typical cystic cavity shape within an injured cord (Baumann et al., 2009; Hejčl et al., 2010).

Complete gel hydration was achieved by allowing the samples to soak in an isotonic solution in a standard incubator at 37 °C and 5 mole % CO_2 atmosphere overnight (Santoro et al., 2011). The swelling equilibrium was rapidly attained within the first hour and, on the other hand, the stability of this material was quite high: it remained stable for weeks before significant degradation by hydrolysis took place (Rossi et al., 2011). As the characteristic time is much longer than the experiment, degradation reactions can be entirely neglected in this work. Therefore, drug flux is controlled only by concentration gradient and diffusion can be considered as Fickian (Crank, 1975). The

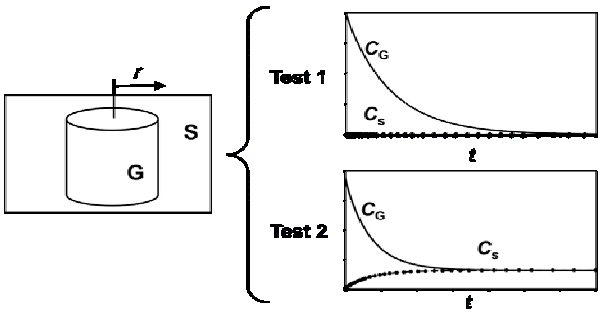

Fig. 1. Schematic representation of the system for cylindrical geometry and of the two experiments assessed. In test 1 $C_S = 0$ due to the continuous replacement of solvent, in test 2 the system reaches the equilibrium condition.

following day, the hydrogels were placed in wells filled with Millipore water (5 mL volume each). At regular intervals (i.e. 1 h, 2 h, 4 h, 6 h, 8 h, 24 h, 30 h, 54 h, and 72 h), the UV absorbance (UV-Vis Diode Array Spectrophotometer (Perkin–Elmer l6 20M), $\lambda = 247$ nm) of the solutions from the wells was measured to assess the fraction released. Some samples had their well solution replaced with Millipore water at certain time intervals (test 1): this procedure is commonly used to best simulate the in vivo release typical of highly vascularised tissues, which are expected to show a fast drug elimination (Fig. 1) (Perale et al., 2009, 2010). Moreover, it substantiates the assumption that the concentration gradient driving force attained a maximum value and was almost constant over the time (Perale et al., 2009). On the other hand, the other samples were left in their solutions without being replaced, thus progressively reducing the concentration gradient until the equilibrium was reached (Fig. 1) with no driving force between the samples and the surrounding solutions (test 2). This procedure is typical of in vitro release tests and low-vascularised organs. All the data of release were used to develop a mathematical model taking into account the key parameters that influence the diffusion phenomena, thus allowing the drug diffusion coefficient D to be estimated.

Where applicable, experimental data were analysed using analysis of variance (ANOVA). Statistical significance was set to $p < 0.05$. The results are presented as mean value ± standard deviation (Tan et al., 2009).

Theoretical

The mathematical model thus developed is based on mass balances, i.e. on fundamental conservation laws. Diffusion is assumed as Fickian and described by means of the second Fick's law with a 1-dimensional model in cylindrical geometry as can be seen in Fig. 1 (Perale et al., 2009, 2010) and shown in Eq. (1). This particular hydrogel shape was chosen as best fitting the average cystic cavity present inside an injured

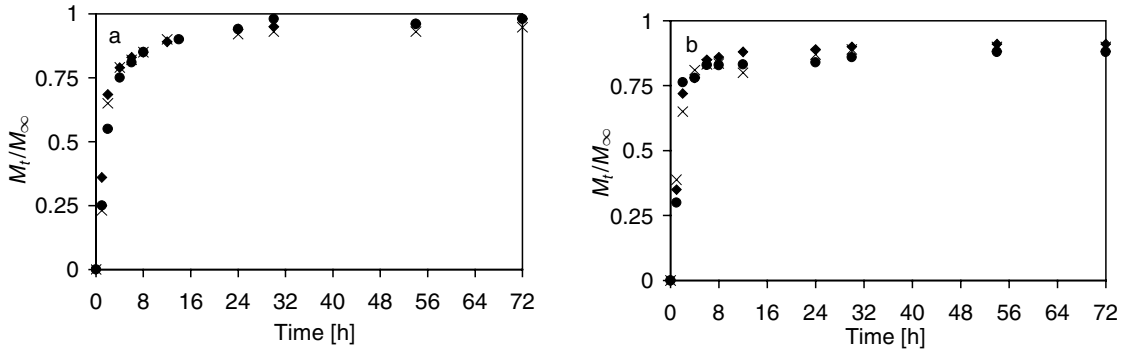

Fig. 2. MP release profiles from all gels (M_t), expressed as a unitary fraction with respect to total loaded mass (M_∞): AC-MP_H (×); AC-MP_M (◆); AC-MP_L (●). Both experiments are shown: a) (left) test 1, b) (right) test 2; standard deviation bars are not plotted (all being ± 7 %).

spinal cord (Baumann et al., 2009; Hejčl et al., 2010). Here the radius (r) is the characteristic dimension of the phenomenon investigated. To take the attainment of equilibrium into account, the status of the equilibrium to be reached for test 2 systems, the increase in drug concentration in the surrounding solution is expressed in Eq. (2). Therefore, the increase referred to above takes place due to the material flux, which takes place on the water/hydrogel surface. Eqs. (5) and (6) represent the boundary conditions for the left and the right border intervals, respectively. The first implies the profile symmetry at the centre (that is, with respect to cylinder axis), while the second represents the equivalence between the material diffusive fluxes at the water/hydrogel surface.

$$\frac{\partial C_\mathrm{G}}{\partial t} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_\mathrm{G}}{\partial r}\right) \tag{1}$$

$$V_\mathrm{S}\frac{\partial C_\mathrm{S}}{\partial t} = k_\mathrm{C}S_\mathrm{exc}\left(C_\mathrm{G} - C_\mathrm{S}\right) \tag{2}$$

$$C_\mathrm{S}\left(t=0\right) = 0 \tag{3}$$

$$C_\mathrm{G}\left(t=0\right) = C_{\mathrm{G},0} = \frac{m_{\mathrm{G},0}}{V_\mathrm{G}} \tag{4}$$

$$\left.\frac{\partial C_\mathrm{G}}{\partial r}\right|_{r=0} = 0 \tag{5}$$

$$-D\left.\frac{\partial C_\mathrm{G}}{\partial r}\right|_{r=R} = k_\mathrm{C}\left(C_\mathrm{G} - C_\mathrm{S}\right) \tag{6}$$

The two mass balance equations involve the mean drug concentration within the hydrogel (C_G), the mean drug concentration in the surrounding solution (C_S), the volume of the solution (V_S), the hydrogel volume (V_G), time (t), hydrogel radius (R), radial variable (r), the drug mass present inside the matrix (m_G), and the exchange interfacial surface (S_exc), i.e. the boundary surface between the gel and the surrounding solution (for simplicity, it can be considered as being only the side surface).

Finally, D represents the diffusion coefficient and k_C the mass transfer coefficient. The mass transfer coefficient is computed using Sherwood number obtained by means of penetration theory (Perale et al., 2009, 2010):

$$Sh = \frac{8}{\pi} = \frac{k_\mathrm{C}2r}{D} \tag{7}$$

During the simulation in test 2, an increase in C_S is described through use of the second mass balance model in order to take the status of equilibrium into account; increase in C_S is simply due to the mass flux which occurs on the water/hydrogel surface. Without water replacement, the concentration gradient will become zero after a certain time. In test 1, a reasonable simplification takes account of the fact that, under in vivo conditions, biological fluids, due to their high clearance, instantly transport the drugs released away from the delivery site. Thus, because the drug removal in vivo is usually much faster than any other phenomenon involved, the external concentration can be considered as equal to zero without losing generality (Perale et al., 2009; Santoro et al., 2011). The (1)–(2) system was calculated numerically assuming the constant gel dimensions (a reasonably valid assumption as degradation phenomena occur at a much slower rate than delivery, and because experiments were carried out on already gelled samples (Rossi et al., 2011)). This assumption also relates to the test 1 system because the aliquot of the solution sampled at each time is substantially smaller than the solution volume.

Results and discussion

The data for MP release from both the experiments performed are reported in Fig. 2, test 1 (a) and test 2 (b), respectively: both plots present the cumulative mass fraction released into the surrounding solution (M_t/M_∞) where the total load in each sample type (i.e. 1/1) was 14.3 μg (AC-MP_H), 4.75 μg (AC-MP_M), and 0.285 μg (AC-MP_L).

Both Figs. 2a and 2b show a rapid initial release of

MP due to the initial concentration gradient. As can be seen in Fig. 2, the initial slope is similar in both tests, both systems are far from equilibrium conditions and therefore unaffected by the eventual potential replacement of the external solution. The rapid initial release of MP takes the form of a burst effect.

This burst effect was more likely caused by: (*i*) drug molecules that were at or near the solvent–hydrogel interface and thus could rapidly penetrate the supernatant solution and (*ii*) drug molecules that found a fast path through large pores of the hydrogel, in contrast to those that had to diffuse through smaller pores, thus partially suffering a constrained molecular motion. After the initial burst, the release became slower and reached an almost steady state condition after about 48 h (plateau). It must be noted that in test 1 all the MP content loaded was released: while this is comprehensible due to the high clearance, it also gave indirect and very important information about the absence of chemical interactions between MP and the hydrogel matrix which can inhibit drug diffusion. Thus, a simple release experiment, like test 1, also revealed the reliability of the couple hydrogel/molecule as a possible drug delivery carrier. Furthermore, the time of complete release of MP which was about 48 h, was fully compatible with medical requirements (Bracken et al., 1997; Cao et al., 2010; Kim et al., 2009).

On the other hand, test 2 (Fig. 2b) showed an incomplete release of MP, due to the equilibrium between hydrogel and the surrounding solution. Although this trend is quite logical, the equilibrium attained gives further information about the MP/hydrogel system. The plateau concentration recorded would be the same if we consider the MP mass dissolved in a solution of the same volume as the volume of the whole system (gel + solution). Thus, with no difference between the equilibrium concentration of hydrogel and the solution, we can also disregard the physical phenomena, e.g. MP adsorption on the hydrogel. In the above considerations, we can assume that all the loaded MP is dissolved in the hydrogel internal medium and is free to diffuse driven by concentration gradients. This is widely valid for both test conditions and it can be explained by considering the ratio between mean gel network mesh size ((7 ± 0.35) nm (Santoro et al., 2011)) and the MP mean hydrodynamic radius ((1.53 ± 0.03) nm (Claußen et al., 2003)): diffusing molecules are not physically bound inside the entangled hydrogel network and are allowed to diffuse with a high free motion (Santoro et al., 2011). Diffusion coefficient D was computed using a least-squares method, minimising the sum of the square of the errors between the experimental and the predicted data .

Fig. 3 shows the comparison between experimental and theoretical rates of release in test 1 with the AC-MP_M gel samples, in terms of mass fraction released into the surrounding solution (M_t/M_∞). The agreement between model prediction and experimental data permits the statment that the model correctly describes the delivery kinetics as it simulates the experimental drug release in good compliance with reality.

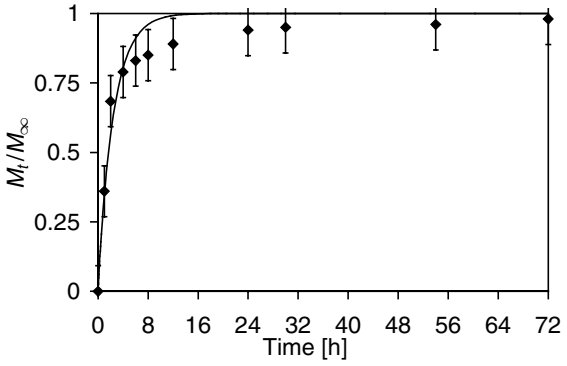

Fig. 3. Comparison between theoretical best fit ($R^2 = 0.95$) and experimental (◆) rates of test 1 release study (AC-MP_M).

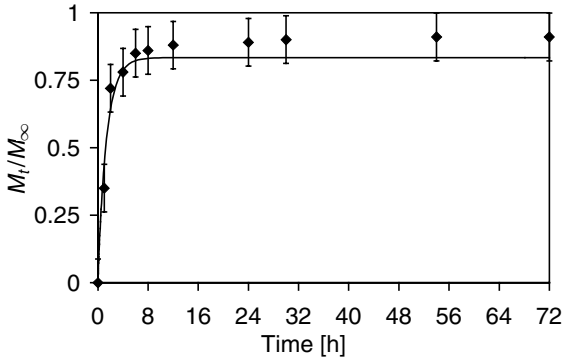

Fig. 4. Comparison between theoretical ($R^2 = 0.96$) and experimental (◆) rates of test 2 release study (AC-MP_M).

As in Fig. 3, Fig. 4 shows the comparison between experimental data and model prediction in test 2 with the AC-MP_M gel samples. Fig. 4 presents the plot of percentage of the drug released versus time.

The same good fit between the experimental data and model predictions was achieved with all the other available data sets, which are not presented in this study. The calculated numerical values of diffusivities are reported in Table 1.

The diffusivity values are all in a very small range between about 4×10^{-9} m^2 s^{-1} and 6×10^{-9} m^2 s^{-1}. Considering the analytical sensitivity, numerical precision and standard deviations, these numbers can easily be considered as absolutely comparable. Furthermore, previous investigations estimated sodium fluorescein (CAS 518-47-8) diffusivity inside the same hydrogel matrix (Santoro et al., 2011) to be about 1.3×10^{-9} m^2 s^{-1}. Indeed, for its steric hindrance (0.8 nm) and molecular mass (376 Da), sodium fluorescein is often

Table 1. Diffusion coefficient of MP in both tests

Test	Sample	$D \cdot 10^9$ [m^2 s^{-1}]
1	AC-MP_L	3.00 ± 0.15
	AC-MP_M	3.89 ± 0.19
	AC-MP_H	3.34 ± 0.17
2	AC-MP_L	5.75 ± 0.28
	AC-MP_M	5.84 ± 0.29
	AC-MP_H	5.56 ± 0.27

used as a drug mimetic, in simulating steroids in particular (Perale et al., 2009; Santoro et al., 2011).

These results confirm the reliability of the model and its assumptions, the correctness of its parametrical estimate and, as expected, irrespective of the experimental set-up or drug concentration.

Conclusions

Agar–Carbomer hydrogel was loaded with methylprednisolone in order to study the kinetics of release of this neuroprotective drug: this system could be a promising tool for spinal cord repair strategies, taking advantage of both the good material ability to mimic central nervous system tissue and the well-known efficacy of the drug. The release kinetics can be characterised by two well-distinguished steps: a rapid initial release followed by a plateau trend. The release experiments were assessed using different drug concentrations and different experimental designs. Irrespective of the concentration or the setting, the drug release was always complete, confirming the absence of chemical and physical interactions between MP and the gel polymeric network. Furthermore, the delivery occurs via a gradient-driven diffusion and its timescale appeared consistent with the specific medical needs. The diffusion process was modelled using second Fick's law, by means of a 1-dimensional model of cylindrical geometry, typical of a cystic cavity along an injured spinal cord. The estimated values of D were significantly similar and comparable with those evaluated in other similar systems, thus confirming the complete understanding of the phenomena and the suitability of the methods adopted here.

Acknowledgements. Supported by Fondazione Cariplo, grant No. 2010/0639. The authors would like to thank Dott. Sacchi (Milan, Italy) for providing them with pharmaceutical grade Carbomer 974P.

Symbols

C_G	drug concentration within hydrogel	μg mL^{-1}
$C_{G,0}$	drug concentration within hydrogel at time $t = 0$	μg mL^{-1}
C_S	drug concentration in solution	μg mL^{-1}
D	diffusion coefficient	m^2 s^{-1}
d	hydrogel sample diameter	cm
k_C	mass transfer coefficient	m s^{-1}
M_t	cumulative mass released at time t	μg
M_∞	cumulative mass released at infinite time	μg
$m_{G,0}$	drug mass present inside matrix	μg
R	coefficient of determination	
r	hydrogel radius	cm
S_{exc}	exchange interfacial surface	cm^2
Sh	Sherwood number	
t	time	s
V_G	volume of hydrogel in hydrated state	cm^3
V_S	volume of solution	cm^3

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