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Cameron Ferris

University of Wollongong, cjf146@uowmail.edu.au

Marc in het Panhuis

University of Wollongong, panhuis@uow.edu.au

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Keywords

hydrogels, b12, gellan, gum, carbon, diffusion, nanotube, vitamin

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Diffusion of vitamin B₁₂ in Gellan Gum-Carbon Nanotube Hydrogels

C.J. Ferris and M. in het Panhuis

Soft Materials Group, School of Chemistry,
University of Wollongong, Wollongong, NSW 2522, Australia. E-mail: panhuis@uow.edu.au

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Keywords – gellan gum; hydrogel; diffusion; conductivity

I. INTRODUCTION

Nanobionics: the merging of biology and electronics using the most recent advances in nanotechnology will play a significant role in the future development of medical implants and prosthetic devices. The realisation of novel material platforms that facilitate nerve cell regeneration, can control muscle cell regrowth, or that act as efficient platforms for endothelial cell growth is envisaged. In addition, the development of artificial muscle fibres for use in wearable prosthetics will benefit from advances in nanotechnology and the development of bio-composite materials.

The field of Bionics has to date been dominated by the use of traditional conductors such as metals and by traditional electromechanical devices such as motors. The advent of organic conductors (such as carbon nanotubes) with the emergence of nanotechnology may revolutionize the field of Bionics, enabling us to more effectively bridge the gap that currently separates the interface between biology and electronics. However, carbon nanotubes (CNTs) alone will not provide the blend of biological, mechanical and electronic properties required for the next generation of Bionics.

CNTs possess unique electronic and mechanical properties¹. A key challenge in exploiting these phenomenal properties is to overcome practical difficulties in processing them from their as-produced state. These difficulties arise as a result of the hydrophobic nature of CNTs and associated strong attractive interactions between adjacent CNTs². Considerable effort is currently being directed towards combining polymers and carbon nanotubes into composites with unprecedented mechanical and electrical properties^{3,4}.

A range of biomaterials have proven suitable hosts for CNTs, and potentially provide the basis of highly effective material platforms for Bionics. Chitosan is used in single-polymer scaffolds, as it is structurally similar to the extracellular matrix component glycosaminoglycans and is biodegradable in humans⁵. Gellan gum is US FDA and European Union (E418) approved for food and medical usage,

and has found wide application as a multifunctional gelling, stabilising and suspending agent as well as an emerging material for tissue engineering applications⁶.

These biopolymers can be processed into hydrogels (a class of highly hydrated polymer materials) which are emerging as a viable scaffold material for tissue engineering applications due to their bio-degradability, process-ability, and similarities to the natural extra-cellular matrix⁵. Physical cross-linking of a biopolymer solution via addition of cross-linking agents such as Ca²⁺ is a well established fabrication method for hydrogels. Previously we applied this technique to gellan gum solutions and gellan gum-CNT dispersions^{7,8}.

We report the formation of hydrogels based on the addition of conducting carbon nanotube fillers to the biopolymers and investigate the diffusion of vitamin B₁₂ in gellan gum and gellan gum-CNT hydrogels.

II. EXPERIMENTAL METHODS

A. Fabrication of gellan gum hydrogels

Solutions of endotoxin-free gellan gum (Gelzan CM, CP Kelco, Lot #7K1383A) in Milli-Q water (mH₂O) ($18 \text{ M}\Omega \text{ cm}^{-1}$) were prepared at concentration of 1.0 % w/v by adding the appropriate mass of gellan gum powder to mH₂O and stirring for ~2hrs at a temperature of ~80°C. To the resultant hot gellan gum solution, a volume of hot 1M CaCl₂ solution (prepared from CaCl₂·2H₂O salt, UNIVAR Lot #712009) was added with continued stirring, so that the final Ca²⁺ concentration in solution was 5 mM. The Ca²⁺ acted as a cross-linking agent, allowing the formation of a hydrogel. The volume of CaCl₂ solution added was small, and consequently did not significantly affect the concentration of gellan in solution. Solutions were then injected into a range of moulds, depending on the purpose of the particular gel, and cooled at room temperature to form a hydrogel.

B. Fabrication of gellan gum-carbon nanotube hydrogels

Single-walled carbon nanotubes (SWNT) (UNIDYM, Lot #P0348) or thin multi-walled carbon nanotubes (MWNT) (Nanocyl SA, Lot #NFL65) were added to hot gellan solutions prior to the addition of Ca²⁺ ions. Carbon nanotubes (CNT) were dispersed in the gellan solutions by probe sonication (Branson Digital Sonifier). The sonolysis regime employed included 10 minutes total sonication time in pulsed mode (0.5 seconds pulse on/off) at an amplitude of 20%. All solutions

were kept in a hot water bath (~80°C) throughout the sonication process to prevent the onset of gelation. CNTs were initially dispersed at a concentration twice that required in the final gel, followed by the addition of an equivalent volume of an unsonicated gellan gum solution to the gellan gum-CNT dispersion. This enabled the formation of a stable hydrogel, and did not appear to adversely affect dispersion quality. Final CNT concentrations ranged from 0.25 – 0.50% w/w. Hydrogels were cross-linked as described previously by the addition of hot CaCl₂ solution to a hot, stirred gellan-CNT dispersion.

C. Electrical characterisation

For the electrical characterisation gellan gum-CNT hydrogels were set on a substrate consisting of two highly porous reticulated vitreous carbon (RVC) electrodes fixed to a glass slide. The RVC electrodes were connected to electrical characterisation equipment consisting of an arbitrary waveform generator (Agilent 33220A) coupled to a digital multimeter (Agilent 34410A). Current-voltage characteristics were measured by applying a triangular DC voltage waveform (0.5 V amplitude, 10 mHz frequency) across hydrogel samples, while current data were recorded at a rate of 2 s⁻¹.

D. Diffusion Studies

Diffusion through gellan and gellan-CNT hydrogels was characterised utilising a method similar to that employed by Li et al to measure diffusion coefficients through alginate and agarose hydrogels⁹. The rate of solute uptake by hydrogel cylinders from a stirred solution of limited volume was determined by measuring the decrease in solute concentration in the surrounding solution over time, allowing the diffusion coefficient to be calculated using an unsteady-state transport model¹⁰. The solute chosen was for this investigation was Vitamin B₁₂ (Sigma-Aldrich, approx. 99%, lot#117K1520). Vitamin B₁₂ was chosen as it is a physiologically relevant molecule that is readily dissolved in water, and its concentration in solution may be monitored easily by absorption at 550nm.

Hydrogel cylinders (length~8cm, diameter = 0.5cm) of various compositions were set in latex tubing. For each hydrogel composition, three identical cylinders were placed in separate 15mL vials containing 13.5mL of a 0.25mg/mL solution of Vitamin B₁₂ in mH₂O. This Vitamin B₁₂ concentration had previously been determined to be the most suitable from preliminary experiments. The vials containing the hydrogel cylinders were then rotated in an orbital shaking water bath (Paton Scientific, model OW1412) at ~120rpm at a constant temperature of 37°C. Over the course of three days, the time previously determined necessary for solute concentrations to reach equilibrium, 200μL aliquots were removed from the surrounding solutions at regular intervals (8 measurements) and placed in 96 well plates. At the conclusion of this period, Vitamin B₁₂ concentration was determined by reading absorption in the wells at 550nm. This was achieved utilising a micro-plate reader (SPECTRAMax Plus 384, Molecular Devices) interfaced to SOFTmax PRO software (Molecular Devices). Concentration of Vitamin B₁₂ at each measurement time was subsequently calculated from a calibration curve.

III. RESULTS AND DISCUSSION

Stable, self-supporting hydrogels (Figure 1) could be easily formed by combining equivalent volumes of an un-sonicated gellan gum solution with a sonicated gellan gum-CNT dispersion prior to addition of the Ca²⁺ cross-linker. The electrical conductivity of the resulting hydrogels was ~10⁻³ S/m in good agreement with our previously reported values⁸.

Figure 1. Photographic image of a self-supporting gellan gum-carbon nanotube hydrogel.

The effect of CNTs on the diffusion of solutes through gellan gum hydrogels was carried out by investigating the transport of Vitamin B₁₂, a physiologically relevant molecule important in cell metabolism through the hydrogels at 37 °C. Diffusion coefficients of Vitamin B₁₂ through cylindrical hydrogels were analysed using the method described by Carmen and Haul¹¹ and Crank¹⁰, and recently employed by Li et al.⁹ to characterise diffusion through agarose and alginate hydrogels.

Equation 1 describes the diffusion of a solute from a stirred solution of limited volume into a solid gel cylinder of length L and radius r , where $L \gg r$, and the diffusion coefficient D is assumed constant.

$$\frac{C_t - C_\infty}{C_0 - C_\infty} = \sum_{n=1}^{\infty} \frac{4\alpha(1+\alpha)}{4 + 4\alpha + \alpha^2 q_n^2} \exp\left(\frac{-Dtq_n^2}{r^2}\right), \quad (1)$$

where C_t , C_∞ and C_0 are the concentrations of the solute in the batch at time t , equilibrium and time = 0, $\alpha = K \times$ (batch solution volume)/(gel solution volume), K is the equilibrium partition coefficient, D is the diffusion coefficient of the solute, t is time, r is the radius of the gel cylinder and q_n are the positive, nonzero root of the equation $\alpha q_n J_0(q_n) + 2J_1(q_n) = 0$, where the J_n are Bessel functions of n^{th} order.

Carmen and Haul¹¹ have tabulated values of q_n as a function of α , and by interpolation one can obtain q for a given value of α characterising a particular sample. By measuring the concentration of the solute in the solution surrounding the hydrogel cylinder over time, the diffusion coefficient can be determined. This is achieved by plotting the following terms:

$$\left[\frac{C_t - C_\infty}{C_0 - C_\infty} \right] \text{versus} \left[\frac{-Dt}{r^2} \right] \quad (2)$$

and calculating the unknown parameter D through non-linear regression⁹ of Equation 2 until the root-mean-square error between the experimental data and theoretical curve is minimised.

After conducting this analysis for a range of hydrogels, the data listed in Table 1 was utilised to obtain a ratio of the diffusion coefficients of Vitamin B₁₂ in each hydrogel (D_{gel}) compared to that in water (D_{water}) at 37°C, which are compared for hydrogels of differing CNT content in Figure 2.

TABLE I. MOLECULAR WEIGHT, RADIUS AND DIFFUSION COEFFICIENT IN WATER (D_{water}) FOR VITAMIN B₁₂.

Molecular Weight (Daltons)	1,300
Stokes radius (Å)	~7.7
D_{water} (cm²/s) at 37°C	5.00×10^{-6}

Even with no added CNTs, the rate of vitamin B₁₂ diffusion through a hydrogel gel is approximately 0.34 times the rate of diffusion in water. This rate of diffusion is similar to that reported for vitamin B₁₂ through alginate hydrogels⁹.

Figure 2 shows that addition of CNTs to the hydrogel significantly decreases the rate of vitamin B₁₂ diffusion. Incorporating 0.25% w/v SWNT or 0.25% w/v MWNT into a gellan gum hydrogel reduces the diffusion coefficient of vitamin B₁₂ in the hydrogel (D_{gel}) from 1.70×10^{-6} cm²/s to 0.70×10^{-6} cm²/s.

Figure 2. Vitamin B₁₂ diffusion as a function of hydrogel type: GG = 1% w/v gellan gum, GG-SW025 = 1% w/v gellan gum and 0.25% w/v SWNT, GG-SW05 = 1% w/v gellan gum and 0.5% w/v SWNT, and GG-MW025 = 1% w/v gellan gum and 0.25% w/v MWNT. All gels were cross-linked with 5 mM Ca²⁺.

These results demonstrate that the addition of CNTs to a hydrogel comes at a cost of restricted solute diffusion through the hydrogel bulk. It is suggested that the greater proportion of dry mass (CNTs) in a gellan gum-CNT hydrogel compared to a

gellan gum hydrogel may be responsible for the observed reduction in D_{gel} .

IV. CONCLUSION

CNTs were incorporated into gellan gum hydrogels in a step towards the development of conducting hydrogels that could potentially entrap and electrically stimulate cells, inducing functional tissue formation. Dispersion of CNTs was successfully achieved using sonication, and stable, self-supporting hydrogels could be formed if un-sonicated gellan was mixed with the sonicated CNT-containing solution prior to gelation. Quantitative measurements undertaken using vitamin B₁₂ revealed that the diffusion coefficient through gellan gum hydrogels was ~0.34 that in water, and addition of CNTs further reduced the rate of diffusion to ~0.14 that in water.

This work contributes towards the development of conducting hydrogel materials for applications in tissue engineering and nanobionics.

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REFERENCES

- [1] R.H. Baughman, A.A. Zakhidov, and W.A. de Heer, "Carbon nanotubes – the route towards applications", *Science*, vol. 297, p 787, 2002.
- [2] M. in het Panhuis, "Carbon nanotubes: enhancing the polymer building blocks for intelligent materials", *J. Mater. Chem.*, vol 16, p 3598, 2006.
- [3] J.N. Coleman, U., Khan, and Y.K. Gun'ko, "Mechanical reinforcement of polymers using carbon nanotubes", *Adv. Mater.*, vol. 18, p 689, 2006.
- [4] N. Grossiord, J. Loos, O. Regev, and C.E. Koning, "Toolbox for dispersing carbon nanotubes into polymers to get conductive nanocomposites", *Chem. Mater.*, vol. 18, p 1089, 2006.
- [5] J.L. Drury, and D.J. Mooney, "Hydrogels for tissue engineering: scaffold design variables and applications", *Biomaterials*, vol. 24, p 4337, 2003.
- [6] A.M. Smith, R.M. Shelton, Y. Perrie, and J.J. Harris, "An initial evaluation of gellan gum as a material for tissue engineering applications", *J. Biomater. Appl.*, vol. 22, p 241, 2007.
- [7] C.J. Ferris and M. in het Panhuis, "Gel-carbon nanotube composites: the effect of carbon nanotubes on gelation and conductivity behaviour", *Soft Matter*, vol. 5, p 1466, 2009.
- [8] C.J. Ferris and M. in het Panhuis, "Conducting bio-materials based on gellan gum hydrogels", *Soft Matter*, vol. 5, p 3430, 2009.
- [9] R.H. Li, D.H. Altreuter, and F.T. Gentile, "Transport Characterisation of hydrogel matrices for cell encapsulation", *Biotechnol. Bioeng.*, vol 50, p 365, 1996.
- [10] J. Crank, 1975. *The mathematics of diffusion*. 2nd edition. Oxford University Press, New York.
- [11] P.C. Carmen, and R.A.W. Haul, "Measurement of diffusion coefficients", *Proc. Roy. Soc. A*, vol 222, p 109, 1954.