TECHNICAL NOTE - Polyvinyl alcohol (PVA) as viable membranes in artificial organ design and development

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Title

Polyvinyl alcohol (PVA) as viable membranes in tissue engineering design and development

Running title

The use of polyvinyl alcohol (PVA) as material of choice in the design and development of artificial membranes for tissue engineering applications

Scientific address

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Title

Polyvinyl alcohol (PVA) as viable membranes in artificial tissue design and development

Abstract

Objectives: To document the permeability and the diffusion coefficient of aqueous polyvinyl alcohol hydrogel membrane to be used in generic artificial tissue engineering applications, using Dextran-FITC as the solute of choice.

Introduction: Polyvinyl alcohol hydrogel has been used in various design geometries to be incorporated in many biomedical applications, particularly involving the encapsulation and the controlled release of specific molecules and particles. Prior to its use in these applications, its fundamental properties, namely permeability and diffusion coefficient, must be ascertained.

Methods: Polyvinyl alcohol hydrogel membrane of specific dimensions will be fabricated, and the rate of diffusion of Dextran-FITC through the membrane will be measured using fluorometer.

Results: Permeability and diffusion coefficient values were determined based on Fick’s Law.

Discussion: The majority of acquired data were consistent with previously published values. Inconsistent results could be due to the change in the experimental protocol when using the experimental apparatus, and/or undetected environmental changes that may affect the fluorescent content of the samples.

Conclusions: Acquired data confirmed that polyvinyl alcohol hydrogel membrane fabricated in the specified dimensions are suitable for use in generic artificial tissue design and development.
Key words

Artificial membrane, Dextran-FITC, PVA, permeability, diffusion coefficient
Introduction

Polyvinyl alcohol (PVA) membranes have long been used in a number of applications, most notably in a number of recent researches involving its uses in biomedical applications, namely encapsulation of hybrid-type artificial organs [1,2,3], controlled release of specific molecules [4,5], targeted drug delivery systems [6,7], enhanced wound dressings [8,9], and various other applications which utilizes its semi-permeability and highly biocompatible characteristics. Nevertheless, prior to its use as the biomaterial of choice in the design and development processes of any artificial tissue fabrication, the exact nature of its physiological properties needs to be ascertained. In this case, the permeability and the diffusion coefficient are the two important parameters that needed to be analyzed. The experimental results will be used in determining the appropriate fabrication technique and design geometry to be used in designing and developing the required tissue engineering applications.

The premise in conducting the study is based on the presumption that if one could place a semi-permeable membrane in between two chambers (Chambers 1 and 2) containing differing concentrations of the same fluid, then the diffusive parameters of the membrane can be determined simply by continuously measuring the changes in solute concentration in the lower-concentration chamber. If one were to wait long enough for equilibrium to be reached, both of the chambers will have the same amount of solutes, and thus concentration levels. The complete concentration profile will then provide the information needed in order to determine the desired parameters based on Fick’s Law.

Nevertheless, this is highly unfeasible due to the long period of time required to record the observations and any related changes. On the other hand, by deriving a model of the underlying diffusion process based on Fick’s Law, one could still predict the diffusive parameters based on data collection over a relatively short period of time. The derivation of the model starts with
constructing material balances on either side of the membrane. The material balance on the higher concentration chamber (Chamber 1) can be shown as

\[ V_1 \frac{dc_1}{dt} = -PA(c_1 - c_2) \]  (1)

where \( V_1 \) is the chamber volume, \( P \) is the permeability, \( A \) is the exposed area of the membrane, \( c_1 \) is the concentration in Chamber 1, and \( c_2 \) is the concentration in Chamber 2. Similarly the material balance in the lower concentration chamber (Chamber 2) can be shown as

\[ V_2 \frac{dc_2}{dt} = PA(c_1 - c_2) \]  (2)

Assuming initial conditions of \( c_1 \) equals \( c_0 \), and \( c_2 \) equals zero, the following mathematical model can be derived, which will be used in determining the permeability, \( P \) and consequently, the diffusion coefficient, \( D \) of the hydrogel membrane.

\[ \ln \left[ 1 - \frac{2c_t}{c_0} \right] = -\frac{2A}{V} Pt \]  (3)

One could quickly observe that if the ratio of \( c_t/c_0 \) is sufficiently small, as is the case if the period of the experiment is shortened, then the left-hand side of the equation will be equal to \(-2c_t/c_0\), according to Taylor’s series. In other words, a linear concentration profile can be expected if the period of conducting the experiment is kept sufficiently short, and the permeability and thus the diffusion coefficient can then be easily determined.

The aim of this technical report is therefore to study the fundamental parameter in the design and development of aqueous PVA hydrogel membrane to be used in generic artificial tissue engineering applications, namely the permeability and the diffusion coefficient, using Dextran-FITC as the solute of choice.
Materials and methods

The methods by which the concentration changes can be measured are varied, and the one chosen for the current study utilizes modified glucose molecules which are tagged with fluorescent material (i.e. Dextran-FITC). The fluorescent levels can be easily measured using a fluorometer, and its levels linearly correlated with the concentration of the glucose molecules in the chamber. A standard concentration curve will need to be constructed such that the concentrations can be calculated from the fluorescence levels based on the linear equation derived from the curve. The derivation of permeability and diffusion coefficient values are based on Fick’s Law, thus requiring the values for a number of parameters (including chamber volumes, membrane aperture, and sample times) in addition to the concentrations.

The methods can be divided into two stages, namely the preparation and the running stages. The preparation stage consists of the preparation of PBS and Dextran-FITC solutions, PVA hydrogel membrane to be tested, and the experimental chamber apparatus (to ensure any foreseeable problems during experimental runs are minimized). In addition, apart from the actual running of the experiment, the running stage also consists of the calculation of permeability and diffusion coefficient for that particular hydrogel membrane used in the experiment.

PBS solutions

To prepare a phosphate buffer saline solution of pH around 7.4, about 7 g of PBS is dissolved in 1 litre of deionised water with continuous stirring. The pH is then measured, and small amounts of PBS will be added until the solution pH reaches 7.4.

Dextran-FITC solutions

Two concentrations were used in the study, namely 100 and 200 µg/ml. The latter is chosen later in the course of the study as to decrease the uncertainties and errors associated with using low concentrations of solute. The 100 µg/ml solution is prepared by mixing 2 mg of Dextran-FITC
with 20 ml of deionised water (or PBS). The amount of Dextran-FITC is doubled in order to produce the 200 µg/ml solution.

Only 12 ml of the solution will be used to fill up one of the chamber in the permeation device, and the rest of the solution is used for preparation of standard Dextran-FITC samples. Each types of the solution is prepared fresh prior to each run of the experiment and is used only once.

**PVA hydrogel membranes**

A 15 wt% PVA solution is prepared from a 30 wt% stock solution. To prepare a 1 ml solution, 0.5 g of the stock solution is mixed with 0.5 ml of deionised water in a tube. Thorough mixing is achieved using an electric mixer, and air bubbles are removed using the vacuum flask in the fume cupboard. The solution should be stored at 4°C until used.

A small volume will be drawn from the tube using a disposable syringe and injected onto a microscope slide. Two cover slips with a thickness of 0.15 mm will be put on both ends of the slide, and another slide will be put on top of the cover slips to produce a thin membrane of fluid.

The UV spot cure system will be used for the cross-linking process, and the exposure time is set at 100 seconds. The separation between the microscope slides arrangement and the tip of the UV system is kept at a distance of about 25 to 30 mm.

**Experimental apparatus**

A two-chamber device (Figure 1), previously developed for generic mass transfer studies, made of Perspex is used in this study. Since Dextran-FITC is chosen as the solute of choice, the transparent chamber walls need to be covered (using aluminium foil) to prevent the degradation of the fluorescence levels. A number of openings in the top part of the chamber were sealed using a type of sealant so as to reduce the effect of evaporation, which will greatly affect the concentration of the samples.
The device will be thoroughly washed before the subsequent experimental run is commenced; to ensure no residual Dextran-FITC is left that may affect the accuracy of the fluorescence readings.

**Permeability studies**

Each of the device components will be thoroughly cleaned and dried, especially the surface of the ports where the membrane will be clamped. The prepared hydrogel membrane will then be mounted, any folds and creases are removed, and the membrane is clamped and placed into position using the four mounting bolts.

12 ml of deionised water (or PBS) is then placed in Chamber 2, and Chamber 1 is filled with 12 ml of Dextran-FITC solution. Both chambers will be stirred continuously on a vertical axis and the ports between which the membrane was clamped were chamfered to enhance mixing near the membrane surface. The exposed membrane area is 0.385 cm$^2$ (0.7 cm radius) and the experiments are conducted at room temperature (22.5 ±1.5°C) over an average run time of 7 hours.

Diffusion of Dextran-FITC, or other solutes for that matter, through the polymer network of the PVA hydrogel follow the Fick’s Law, and can be expressed in the following form:

$$\ln\left[1 - \frac{2c_t}{c_0}\right] = -\frac{2A}{V}Pt \quad (4)$$

where $c_t$ is the concentration of the solute in Chamber 2 at time $t$, $c_0$ is the initial concentration of the solute in Chamber 1, $t$ is the diffusion time in seconds, $A$ is the exposed membrane area (0.385 cm$^2$), $V$ is the volume of the chamber (12 ml), and $P$ is the permeability of the hydrogel membrane. The diffusion coefficient is related to the permeability using the following relationship:

$$P = \frac{D}{k\delta} \quad (5)$$
where $D$ is the diffusion coefficient of the membrane, $k$ is the partition coefficient of the solute, and $\delta$ is the membrane thickness at the end of the experiment. The partition coefficient of Dextran-FITC approximately equals to 1 [10]. The permeability and thus the diffusion coefficient could then be calculated by measuring the slope of the linear portion in the plot of equation (1). A highly linear dependence of $\ln \left(1 - \frac{2c_t}{c_0}\right)$ against $t$ could be seen when glucose molecules are passed through the PVA hydrogel membrane.

*Experimental protocol*

Samples are taken from Chamber 1 only at the beginning ($t_0$) and the end ($t_f$) of the run, whereas samples are taken from Chamber 2 at $t_0$, $t_f$, and at a number of specified intermediate times. Using a pipette and a clean tip, 1 ml of sample is taken at each specified instances and is placed in a microtube. 1 ml of deionised water (or PBS) is added to the respective chamber using a clean tip to restore the volume.

The microtubes are quickly placed in a rack and kept in the freezer overnight, alongside the standard concentration samples. The fluorescence level for each of the samples is read the following day. Three 0.2 ml samples from each microtubes will be drawn and placed in a fluorescence counting plate. The frequency range of the fluorometer is set at the default setting of 485-538 µm.

*Results*

The outcome of the study is entirely dependent on the fluorescence readings of the samples. Thus it is of utmost importance that the construction of the standard curve, from which the concentration of the sample is determined, produces consistent and reproducible results. Figure 2 shows the standard curves for both types of solvents, i.e. water and PBS.

The mean R-squared value for water is 0.99 (± 0.0049), while the corresponding value for using PBS is 0.98 (± 0.0022). Although an R-squared value is simply a measure of the predictability of
the next data points (with 0 being impossible to predict, and 1 being the most predictable) based on the construction of the most fit linear equation between the two variables, the author is of the opinion that it can be still be used as a measure of linearity to a certain degree. Therefore it has been shown that the relationship between the two variables may be considered linear within the range of these concentrations, for both water and PBS, and hence could be used for the purpose of determining the concentration of the samples.

An example plot of concentration when using water as the solvent of choice (from Run 8) is shown in Figure 3. Figure 4 shows the plot of \( \ln (1 - 2c_t/c_0)^* \) against \( t \) which is used in determining the permeability and diffusion coefficient of the membrane. The asterisk is included to indicate that the value has been multiplied by a factor of 100,000 as to give a much more precise slope value, which is used in the calculation for permeability. The permeability and thus diffusion coefficient can then be calculated using equations (1) and (2). The calculation for determining \( P \) and \( D \) is shown below, where \( V \) is 12 ml, \( A \) is 0.385 cm\(^2\), and \( \delta_{\text{water}} \) is 0.015 cm.

Equating the \( m \)-part of equation (1) to the slope of the graph in Figure 4 (removing the multiplication factor by multiplying the slope value with \( 10^{-5} \)):

\[
\frac{2A}{V} P = 3.91 \times 10^{-7}
\]

Substituting the values of \( V \) and \( A \) gives the permeability of the hydrogel membrane:

\[
P = \frac{(3.91 \times 10^{-7})(12)}{2 \times 0.385} = 6.10 \times 10^{-6} \text{ cm/sec}
\]

Finally by substituting the value of \( P \) and \( \delta \) in equation (2), the diffusion coefficient of the membrane is determined:

\[
D = (6.10 \times 10^{-6})(0.015) = 0.91 \times 10^{-7} \text{ cm}^2 / \text{sec}
\]
Similar calculation steps and parameter values are also used for determining the permeability and diffusion coefficient when using PBS as the solvent of choice.

Table 1 summarizes the values for a selected number of parameters, namely the initial and final concentrations of Chamber 1, final concentrations of Chamber 2, and the permeabilities and diffusion coefficients of the hydrogel membrane in each of the experimental runs employed in this report.

Discussion

In accordance with the objective of this study, the calculated values of permeability and diffusion coefficient of the membrane will be compared with expected values as found in the literature. Hickey and Peppas [10], for example, used 10 and 15 wt% PVA hydrogel membranes and studied its diffusion coefficient using theophylline and Dextran-FITC as part of their diffusive studies, which was prepared using the freezing/thawing technique. Their studies produced a range of values for the diffusion coefficient, depending on the degree of crystallinity and the equilibrium swelling ratio. Although the diffusion coefficient ranges from $1.70 \times 10^{-8}$ cm$^2$/sec to $5.28 \times 10^{-8}$ cm$^2$/sec, the range is still bound within the same order of magnitude, and thus the author believed that these values are still relevant to be used as a reference. These values are calculated when using water as the solvent.

From Table 1, the values calculated from the study differ within an order of magnitude compared to values taken from previous studies [10,11]. Although the solute and the solvent used in the experiments are exactly the same, there are many differences in the set-up of the experiment which may directly affect the outcome of the results. For example, the degree of crystallinity and the equilibrium swelling ratio are not determined in the current study, and thus making it difficult to place the calculated value within the range of previously determined diffusion coefficient values. These diffusion coefficient values seem to increase non-linearly with an increase in equilibrium swelling ratio, while the degree of crystallinity stays reasonably stable.
In the author’s opinion, one of the fundamental issues that needs to be considered before the difference in the calculated diffusion coefficient values can be analyzed, concerns the issue of consistency in conducting the experiments. If one were to look at Table 1, there is an increase in the final concentration of Chamber 1 \((c_f)\) when using water as the solvent (Runs 6 to 10). This is obviously counter-intuitive, since the concentration should obviously drop, corresponding to an increase in the concentration in Chamber 2. Even when there is a drop of concentration in Chamber 1 when using PBS, the drop does not closely correspond to the increase of concentration in Chamber 2.

There is also a significant difference in the amount of fluorescence obtained from Dextran-FITC samples between the two solvent types used (Figure 2). When using water as the solvent, there is a decrease of up to three times the amount of fluorescence obtained from samples using PBS. Due to the constraints of time, this phenomenon has not been investigated, and it could well play a role in explaining the inconsistencies found in the results.

In addition, there is another inconsistency that is worth noting for the purpose of future investigations. This concerns the thickness of the hydrogel membrane at the conclusion of the experimental runs. In a number of occasions where the thickness is measured post-experimentally, the value is found to be changing when using water as the solvent, whereas it stays the same when using PBS. The thickness of the membrane almost invariably increases at the end of the experimental run when using water; up to two times the initial thickness. This obviously impacts greatly with the dynamics of the diffusion itself, and thus the accuracy of the calculation used in determining the permeability and diffusion coefficient could not be ascertained. It is due to this fact that the initial membrane thickness is used in the calculation instead, since this is common to all of the membranes, and the consistency of the value is assured by using the cover slip as the separator between the microscope slides during the preparation of the membranes.
Conclusions

Although the experimental protocol used in conducting these experiments may have been sufficiently altered to obtain the diffusion coefficient for a given membrane, there are still certain inexplicable aspects of the results that may render the calculated values less useful and relevant. It is therefore highly recommended that these inconsistencies be investigated fully, particularly with regards to the design and fabrication of the experimental chambers, before the protocol can be used in any permeability-related studies in the future. Nevertheless, from these very limited data, it may be safely concluded that PVA is satisfactorily suitable to be used in the design and development of artificial membranes in various tissue engineering studies.

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Table 1 Measured and calculated values for selected parameters. Runs 6 to 10 uses water as the solvent, while Runs 11 to 13 uses PBS. Runs 6 and 8 uses 100 µg/ml Dextran-FITC, while the rest of the runs uses 200 µg/ml Dextran-FITC.

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<th>Experimental runs</th>
<th>Chamber 1 (µg/ml)</th>
<th>Chamber 2 (µg/ml)</th>
<th>Permeability (10⁻⁶ cm/sec)</th>
<th>Diff. coefficient (10⁻⁷ cm²/sec)</th>
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<td>c₀</td>
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<td><strong>Average</strong></td>
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<td><strong>6.2 (±0.65)</strong></td>
<td><strong>0.9 (±0.98)</strong></td>
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<td><strong>1.4 (±0.16)</strong></td>
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Legends for Figures

Figure 1 The two-chamber device used in the experiments (a), and the schematic diagram of the device (b). Dotted lines indicate the clamped membrane.

Figure 2 Standard curves when using water (white) and PBS (black) as the solvent.

Figure 3 Concentration curve of Dextran-FITC in water.

Figure 4 Plot of ln(1 – 2c/tc0) for Dextran-FITC in water. The slope and the R-squared values are shown.
Acknowledgements

NAK and RMG would like to thank the University of Malaya, Malaysia and the Public Services Department, Malaysia for financial grants towards the completion of their postgraduate studies of which this report is part of.
The two-chamber device used in the experiments (a), and the schematic diagram of the device (b).

Dotted lines indicate the clamped membrane.
Standard curves when using water (white) and PBS (black) as the solvent.

160x76mm (300 x 300 DPI)
Concentration curve of Dextran-FITC in water.
160x76mm (300 x 300 DPI)
Plot of $\ln(1 - 2ct/c_0)$ for Dextran-FITC in water. The slope and the R-squared values are shown.