Estimating glucose diffusion coefficient of membranes for tissue engineering applications using Fick’s First Law

D N H Pg Sulaiman, H Suhaimi* and N Shamsuddin

*Faculty of Integrated Technologies, Universiti Brunei Darussalam, Jalan Tungku Link BE1410, Brunei Darussalam

Abstract. The idea of growing artificial tissues in bioreactors such as hollow fibre membrane bioreactors (HFMBs) has started years ago and preparation of biocompatible porous membranes and scaffolds has been attempted extensively. There also have been several studies on modelling glucose transport processes in HFMBs. However, there is little information available that discusses specifically the glucose diffusivity across tissue engineering membranes or scaffolds and, importantly, its dependence on the properties of the materials (i.e., membrane and scaffold). Therefore, the objective of this study is to deduce the glucose diffusivity across different membranes. Using Fick’s law and a diffusion cell in this study, we have determined glucose diffusion coefficients for two different membranes namely cellulose nitrate (CN) and polyvinylidene fluoride (PVDF). These membranes possessed similar pore size with varying thickness and were saturated with water and cell culture medium (CCM). The diffusion experiments were conducted in a thermostated water bath at either 27 or 37 ± 1°C. It was observed that PVDF membrane with greater thickness has a lower diffusivity than CN membrane when both were saturated in CCM and water at 37°C. From the results, we derive the significance of the decrease of diffusion coefficient with increasing thickness of the membranes.

1. Introduction

Tissue engineering (TE) aims to merge cells, scaffolds and biologically active molecules in vitro to become functional tissues and eventually organs [1,2]. The effectiveness of TE is determined primarily by the biological signaling molecules such as the growth factors and also the scaffolds which enable cells to grow into a tissue [1]. These scaffolds are made from biomaterials which performs as a temporary skeletal frame that mimics the properties of extracellular matrix (ECM), such as mechanical support, cell adhesion, cell proliferation and cell differentiation [3–8]. There are a number of polymers used to construct TE scaffolds, such as collagen, silk fibroin and gelatin, which are categorised as natural polymers [9–12]. Meanwhile, there are also synthetic polymers such as polycaprolactone, polyethylene glycol, polylactic acid, polyvinyl alcohol and polyurethane [7–9,13–15].

There are several factors hindering the formation of tissue growth in vitro such as the lack of a natural capillary network that functions to absorb nutrients (e.g. glucose and oxygen) and eliminate the unwanted products (e.g. lactic acid) [16,17]. As a result, the tissue grown could not fully mimic the native tissue found in vivo. The shortfall of the nutrients being absorbed via diffusion process could lead to an irregular integration between the scaffold and native tissue or even implant death [18]. The availability of these nutrients is crucial as several factors play a major role on the success rate of tissue-engineered constructs. Amongst these factors include cell differentiation, survival and proliferation. In contrast to oxygen which has been extensively studied over the years [19–23], there is limited data...
available on the diffusion of glucose in porous membranes and scaffolds saturated in cell culture media (CCM). Most diffusion coefficient data available in literature are for cases where these materials are saturated with water at ambient conditions only. However, the cell/tissue culture experiments are typically conducted at 37-38°C and the materials are saturated with CCM.

The aim of this paper is therefore to quantify the relationship between diffusion coefficient and membrane morphology by engaging typical membrane materials of same pore size with varying thickness for tissue engineering and relating the diffusivity values to the quantitative information of the morphology of the membrane materials. Please note that although the materials chosen for this study are designed for tissue engineering purposes, they were not seeded with any biological cells yet at the time the experiments were conducted. This is because this study is only aimed at quantifying simple passive diffusion of glucose through the materials.

2. Methodology

2.1. Materials and methods
For this study, the diffusion coefficient of glucose was determined across tissue engineering membranes saturated in water and CCM at two different temperatures by engaging the diffusion cell technique.

2.2. Membranes
Two types of membrane, as summarized in Table 1, were employed in this experiment. Prior to conducting diffusion experiments, the membranes were pre-treated by soaking in deionized water for 24 hours to ensure removal of any remaining preservatives on the membrane surface.

| Membrane                    | Pore size (µm) | Thickness (µm) | Manufacturer                                      |
|-----------------------------|----------------|----------------|--------------------------------------------------|
| Cellulose nitrate (CN)      | 0.45           | 115.0          | Millipore UK Ltd (Watford, UK)                    |
| Polyvinylidene fluoride (PVDF) | 0.45          | 121.3          | Supplied by Universiti Teknologi PETRONAS, Malaysia |

2.3. Other materials
Dulbecco’s Modified Eagle Medium (DMEM) was used as the CCM and the glucose was of analytical grade powder D-glucose-anhydrous of molecular weight 180.16 g/mol. Both materials were acquired from Thermo Fisher Scientific, UK.

2.4. Diffusion cell for measurement of glucose diffusivity
To measure the diffusion coefficients of glucose, two acrylic rectangular diffusion cells were constructed, similar to those described by Suhaimi et al. [19]. The two cells were connected together by tightly screwing the half chambers with rubber gaskets attached in between to avoid leakage. As for the membrane, it was fixed in between the rubber gasket. Each cell has an internal geometry of length 45 mm × height 45 mm × width 20 mm, both having the same volume of 50 mL. The two chambers are identified as the donor and receptor phase. Each chamber was filled with either CCM or water and the donor phase also contained glucose solution. Figure 1 shows the schematic diagram of the diffusion cell while Figure shows the front and side view of the assembled diffusion cell.
Glucose powder was first pre-mixed with CCM/water in a beaker before the start of the experiment. Before assembling the apparatus, the pure solution of water/CCM (receptor phase) and glucose mixed with water/CCM (donor phase) were pre-heated in a thermostated water bath at either 27 or 37 ± 1 °C, allowing the solutions to equilibrate for 60 minutes. After assembling the apparatus, the diffusion cell was placed in a thermostated water bath at either 27 or 37 ± 1 °C for the entire duration of the experiment. The temperature of the water bath was constantly monitored using a thermocouple. As shown in Equation (1), Fick’s first law was used to deduce the diffusion coefficient of glucose across membranes.

\[ J = -D \frac{\partial C}{\partial z} \]  

where \( J \) is the mass flux, \( D \) is the diffusion coefficient of the solute, \( C \) is the concentration of the diffusing solute and \( z \) is the diffusion length. By taking into account of the obstruction effects resulting from the diffusion through membranes, these properties are comprised of the effective diffusion coefficient of the material [19,24] which can be seen as
\[ J = -D_e \frac{\partial C}{\partial z} \] (2)

By assuming that the volume was unchanged, Equation (2) was converted to equation (3) as shown below

\[ V_d \frac{\partial C_d}{\partial t} = -D_e A \frac{C_d - C_r}{l} \] (3)

where \( l \) is the thickness of membrane, \( A \) is the area of membrane, \( D_e \) is the effective diffusion coefficient of the material, and \( V_d \) is the volume of the donor phase. With the use of Equation (3), the glucose diffusion coefficients were calculated by measuring the concentration of glucose both in the donor and receptor phase at various times.

2.5. Brix refractometer
A portable brix refractometer was used to monitor the change in glucose concentration over time. Each chamber was filled with 50 mL of water/CCM. The donor phase also contained 8 mg/mL of glucose solution. Samples from both donor and receptor phase were taken using a plastic dropper at intervals of 1 hour until equilibrium was established. A few drops from the samples were deposited onto the main prism of the refractometer to be analysed. To ensure constant volume throughout the duration of the experiment, the samples were immediately poured back into the chambers after analysing. All experiments were conducted in duplicate. The volume loss for each chamber remains consistent for every sample, thus the issue of keeping the volume constant can be ignored.

3. Results and discussions

3.1. Membrane characterization
Scanning electron microscopy (SEM) was utilized to study the microstructures of the membrane. The dry samples were coated with carbon and placed on a sample stand. Figure 3 presents the cross-sectional images of CN and PVDF membrane. The difference in thickness of both membranes attributes to the diffusivity values presented in Table 2.

![SEM micrographs showing the cross-sectional images of (a) CN membrane and (b) PVDF membrane.](image)

3.2. Glucose diffusion analysis
The purpose of employing different types of membranes with the same pore size and varying thickness is to analyse the effect it has on the glucose diffusivities. As mentioned earlier, the diffusion experiments were conducted in both media; water and CCM at two different temperatures as the combination of these
two would correctly present tissue engineering-related experiments. Figure 4 shows the typical temporal change in the concentration of glucose for both donor and receptor phases.

![Figure 4](image1.png)

(a) Donor phase at 37°C
(b) Receptor phase at 37°C
(c) Donor phase at 27°C
(d) Receptor phase at 27°C

**Figure 4.** Glucose diffusion experiment with (a) 8 mg/mL of glucose for CN membrane saturated in water at 27 and 37 ± 1 °C and (b) 8 mg/mL of glucose for CN membrane saturated in CCM at 27 and 37 ± 1 °C.

As expected, the effective diffusion coefficient is higher for CN membrane due to its thickness. It can be seen from the cross-sectional image in Figure 3(a). This is also reflected in the diffusion coefficient values shown in Table 2 where CN membrane with thickness of 115 µm has a larger glucose diffusivity value while PVDF membrane with thickness of 121.3 µm has a smaller glucose diffusivity value. This shows that the corresponding diffusivity value decreases with increasing thickness of the membrane. This is true independent of the media used. It can also be seen that the results for diffusion experiments for CN membrane saturated in water and CCM are different where the diffusivity values in CCM are higher than in water. This may be attributed to the presence of other molecules in CCM which could affect the diffusion process. It is also worth pointing out that the effective diffusion coefficient for CN membrane increases from 27°C to 37°C. This is true for both media (i.e., water and CCM) and can be attributed to the increased in kinetic energy of the glucose molecules at a higher temperature and therefore a decreased in viscosity. The effective diffusion coefficients obtained in the present study (Table 2) is within the range of other reported values by Wang et al. [25] and Boss et al. [26] where the effective diffusion coefficients of glucose saturated in water at 37°C using hydroxypropyl chitosan (HPCTS) crosslinked with gelatin (GEL) and chondroitin sulphate (CS) scaffold and asymmetric alumina membrane, were found to be 1.16 x 10⁻¹⁰ m²/s and 1.39 x 10⁻¹⁰ m²/s, respectively. In more recent studies by Selifonov and Tuchin [27] and Carvalho et al. [28], the effective glucose diffusion coefficients were found to be 5.4 x 10⁻¹⁰ m²/s and 4.4 x 10⁻¹¹ m²/s, in biological tissues, respectively.

As the results from this study presents part of the on-going work, there are still some results that are yet to be determined as can be seen in Table 2. The presented results can pave a way for conducting more diffusion experiments with different membrane materials of either the same pore size with varying thickness or materials with different pore morphological structures.
Table 2. The effective diffusion coefficients of glucose through membranes saturated in water and CCM.

| Membrane | Pore size (μm) | Thickness (μm) | Effective diffusion coefficient (m²/s) |
|----------|---------------|---------------|--------------------------------------|
|          |               |               | Water at 27°C | Water at 37°C | CCM at 27°C | CCM at 37°C |
| CN       | 0.45          | 115.0         | 3.67 × 10⁻¹⁰ | 4.71 × 10⁻¹⁰ | 7.33 × 10⁻¹⁰ | 9.17 × 10⁻¹⁰ |
| PVDF     | 0.45          | 121.3         | N/A          | 5.52 × 10⁻¹¹ | N/A          | 9.67 × 10⁻¹¹ |

4. Conclusion

Two different membranes (CN and PVDF) of the same pore size and different thickness were employed in this study. A simple diffusion cell was developed for the purpose of monitoring the diffusion process. The objective of selecting membranes of different thickness was to observe the effect it has on the diffusion process. The results showed that the effective diffusion coefficient decreases with increasing thickness of both membranes for both water and CCM at 37°C, from 4.71 × 10⁻¹⁰ m²/s to 5.52 × 10⁻¹¹ m²/s at 27°C and 3.67 × 10⁻¹⁰ m²/s to 7.33 × 10⁻¹⁰ m²/s at 37°C, respectively. It can also be observed that the diffusivity of glucose in CN membrane saturated with CCM increases at a given temperature from 3.67 × 10⁻¹⁰ m²/s to 7.33 × 10⁻¹⁰ m²/s at 27°C and 37°C, respectively. This could be due to the presence of other molecules present in CCM. The possibility of different types of culture media used indicating different components present in the media leading to different diffusivity values cannot be ruled out. Although there are still missing results that are yet to be determined, the results presented in this study are encouraging and could be useful for future work. In addition, it is worth noting that other factors such as membrane types and surface roughness can be considered in future studies.

References

[1] Berardi Editor A C 2018 Extracellular matrix for tissue engineering and biomaterials Stem Cell Biology and Regenerative Medicine 4-20
[2] Katari R, Peloso A and Orlando G 2015 Tissue engineering and regenerative medicine: Semantic considerations for an evolving paradigm Front Bioeng Biotechnol 1–6
[3] Bose S, Vahabzadeh S and Bandyopadhyay A 2013 Mater Today 16 496–504
[4] Rezwan K, Chen Q Z, Blaker J J and Boccaccini A R 2006 Biomaterials 27 3413–31
[5] Hutmacher D W 2000 Biomater Silver Jubil Compend 21 175–89
[6] Li X, Cui R, Sun L, Aifantis K E, Fan Y, and Feng Q 2014 Int J Polym Sci 2014
[7] Inamuddin I 2015 Green polymer composites technology: Properties and applications, by Inamuddin 1-591
[8] Ng W L, Chua C K and Shen Y 2019 Progress in Polymer Science. Elsevier Ltd 101145
[9] Atrian M, Kharazia M, Emadi R and Alihosseini F 2019 Appl Clay Sci 174 90–9
[10] Tal H, Moses O, Kozlovsky A and Nemcovsky C 2012 Bioreorbable collagen membranes for guided bone regeneration Bone Regeneration 111-32
[11] Lee S-W and Kim S-G 2014 Maxillofac Plast Reconstr Surg 36 239–46
[12] Kim H-W, Song J-H and Kim H-E 2005 Adv Funct Mater 15 1988–94
[13] Kharazia M, Fathi MH and Edris H 2013 J Mech Behav Biomed Mater 24 9–20
[14] Wang J, Shang P, Shi W and Cui X 2016 Commun Nonlinear Sci Numer Simul 37 115–24
[15] Zhang Y, Liu W, Shao W and Yang Y 2013 Geotext Geomembranes 37 10–5
[16] Suahimi H and Bhusan Das D 2016 Rev Chem Eng 32 629–50
[17] Li S-T, Liu Y, Zhou Q, Lue R-F, Song L and Dong S-W 2014 A Tissue Eng Part C Methods 20 205–14
[18] Moroni L, Schrooten J, Truckenmüller R, Sohier J and Blitterswijk CA 2015 Van tissue engineering : An introduction Second Edi. Tissue Engineering. Elsevier Inc. 1–21
[19] Suhaimi H, Wang S, Thornton T and Das D B 2015 Chem Eng Sci 126 244–56
[20] Malda J, Woodfield T B F, Van Der Vloodt F, Kooy F K, Martens D E and Tramper J 2004 Biomaterials 25 5773–80
[21] Guaccio A, Borselli C, Oliviero O and Netti P A 2008 Biomaterials 29 1484–93
[22] Ellis S J, Velayutham M, Sendhil Velan S, Petersen E F, Zweier J L and Kuppusamy P 2001 Magn Reson Med 46 819–26
[23] Kellner K, Liebsch G, Klimant I, Wolfbeis O S, Blunk T and Schulz M B 2002 Biotechnol Bioeng 80 73–83
[24] Gutenwik J, Nilsson B and Axelsson A 2004 Biochem Eng J 19 1–7
[25] Wang S, Liu W, Han B and Yang L 2009 Appl Surf Sci - APPL SURF SCI 255 8701–5
[26] Boss C, Meurville E, Sallese JM and Ryser P 2012 J Memb Sci 401–402 217–21
[27] Selifonov AA and Tuchin V V 2020 J Opt Technol 87 168–74
[28] Carvalho S, Gueiral N, Nogueira E, Henrique R, Oliveira L and Tuchin V V 2017 J Biomed Opt 22 1–12