

Glucose diffusivity in cell-seeded tissue engineering scaffolds

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Abstract

Objective To determine the effective glucose diffusion coefficient in cell-seeded porous scaffolds to understand the importance of nutrient diffusion in tissue engineering bioreactors.

Results Cell growth changed the morphological structure of the scaffolds decreasing the effective pore space and, inevitably, decreasing the effective glucose diffusivity in the chosen scaffolds, namely, collagen, poly(L-lactide) and poly(caprolactone) scaffolds from 3.7×10^{-9} to 3.2×10^{-9} m²/s, 1.4×10^{-10} to 9.1×10^{-11} m²/s and 1.8×10^{-10} to 1.3×10^{-10} m²/s, respectively.

Conclusions The presence of cells over time during cell culture reduces the mobility of glucose. The results can predict the glucose concentration profiles in thick engineered tissues.

Keywords Diffusion coefficient · Glucose · Osteoblast cell · Scaffold · Tissue engineering

Introduction

The main goal of tissue engineering is to design, construct, regenerate and repair damaged tissues in the human body (Abdullah et al. 2009; Ahn et al. 2014).

Many tissue engineering scaffolds have emerged to mimic extracellular matrices (ECM) that exhibit a biologically-induced stable environment and therefore should promote cell and tissue growth as well as providing mechanical support (Daniele et al. 2014). They play a crucial role in the reconstruction of diseased tissues due to their porous, biocompatible and non-toxic characteristics. They also must have an interconnected porous network for easy access of nutrients into the cells and removal of metabolic wastes such as lactate from the cells (Ahn et al. 2014). As the amount of interconnected pores varies in different tissue engineering scaffolds, the nutrient diffusivity in these scaffolds may also vary (Suhaimi et al. 2015).

In general, the solutes for cell growth (e.g., nutrients and O₂) are transported into the scaffold and into the cells by diffusion. The morphological structure of the scaffolds (e.g., pore size and shape distribution, average porosity, tortuosity, and others) has a significant effect on solute transport processes (Wu et al. 2010; Park et al. 2014; Chao and Das 2015). For example, increasing the cell mass grown in scaffolds decreases the O₂ diffusivity with increasing tissue formation within a tissue engineering scaffold (Kang et al. 2011).

In principle, the presence of cells should affect solute diffusivity in scaffolds by exerting contractile forces on the scaffolds which is then dependent on the cell and scaffold type. For example, fibroblasts and

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mesenchymal stem cells exert contractile forces on collagenous scaffolds (Brown et al. 2002). Awad et al. (2000) and Leddy et al. (2004) reported the effects of changes in scaffold material leading to decreased diffusivity as a result of contractile forces exerted on the scaffold by cells.

In contrast to O₂ diffusivity (Bettinger et al. 2006; Kang et al. 2011; Cheema et al. 2012; Fiedler et al. 2014), there are only limited studies on nutrient diffusivity, especially glucose, within cell-seeded tissue engineering scaffolds. In addressing this issue, we report the glucose diffusivity of tissue engineering scaffolds seeded with human osteoblast cells in cell culture media at 37 °C. These scaffolds were used in our previous work (Suhaimi et al. 2015) but were not seeded with cells as that work aimed only at quantifying passive diffusion of glucose through the materials, i.e., the relationship between glucose diffusion with different amount of connected pores and pore morphology in different tissue engineering scaffolds. In contrast, the goal of the present work was to quantify the diffusive properties of cell-seeded scaffolds and compare with our previous results of non-seeded tissue engineering scaffolds. We show how glucose diffusivity changes with morphological changes of the scaffolds and with cell culture time. Specifically, the glucose diffusion coefficient decreases with increasing cell mass grown on the surface and inside the scaffolds.

Materials and methods

Materials

Human osteoblast HOSTE85 cell line [European Collection of Cell Culture (ECACC), UK] was donated by the Centre for Biological Engineering, Loughborough University. The cell culture medium used was Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % (v/v) foetal bovine serum (FBS) and 2 % (v/v) non-essential amino acids (NEAA). Poly(caprolactone) (PCL), poly(L-lactide) (PLLA) and collagen scaffolds used. Initial porosity of the materials (i.e., before cells were seeded) was evaluated by a pycnometric method, as described in Suhaimi et al. (2015). Glucose (D-glucose-anhydrous) was of analytical grade.

Pore size distribution determination and morphological structure of scaffolds

Pore structures and distribution as well as the morphological structure of the scaffold materials were observed by a scanning electron microscopy (SEM). The cell culture medium was removed and the scaffold specimens were washed with phosphate buffered saline (PBS) to discard any remaining DMEM. The scaffolds were then left to dry inside a class II biological safety cabinet before the SEM analysis. The dry specimens were coated with carbon for 120 s by a sputter coater. The images were taken at a voltage of 5 keV. The minimum, mean and maximum pore sizes of the scaffolds were determined using the ImageJ version 1.48 software (Wayne Rasband, National Institute of Mental Health, USA) where the SEM images were uploaded. Briefly, lines were drawn between the pores and measurements were tracked and recorded by the software (Suhaimi et al. 2015).

Cell culture and seeding

Prior to cell seeding, both PCL and PLLA scaffolds were pre-wetted in 20 % (v/v) ethanol for 30 min followed by washing with DMEM twice in another 30 min. Collagen scaffolds were highly purified and could therefore be seeded in the dry state without previous washing with DMEM. This procedure was carried out after sterilizing the class II biological safety cabinet by exposure to ultraviolet (UV) light for 30 min.

HOSTE85 human osteoblast cells were cultured in DMEM supplemented with 10 % (v/v) FBS and 2 % (v/v) NEAA at 37 °C and 5 % CO₂/95 % air (v/v) in a humidified incubator. The medium was changed every 2 days and the HOSTE85 cells were detached using 0.25 % Trypsin/0.1 % EDTA followed by re-suspending in supplemented DMEM (Fig. 1). After pre-wetting with 20 % (v/v) ethanol and washing both the PCL and PLLA scaffolds twice with DMEM, 1 ml HOSTE85 cell suspension, containing 1.2×10^5 cells and 1.5×10^5 cells, was seeded onto both the PCL and PLLA scaffolds in a 16-well plate, respectively. As for collagen scaffolds, 1 ml HOSTE85 cell suspension containing 6×10^5 cells was seeded into collagen in the dry state, also in a 16-well plate. The cell suspension was also added into an empty well (no scaffold) of the 16-well plate as control. After seeding,

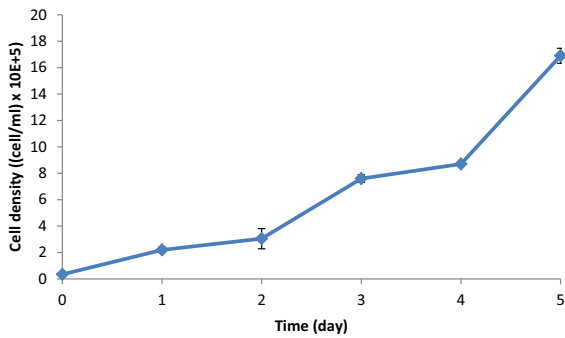


Fig. 1 The cell growth curve for HOSTE85 as a function of time where the doubling time of HOSTE85 cells is 1.49 days. The cells were cultured for 5 days and cell number was calculated on each day using a haemocytometer where trypan blue was added into the cell suspension to differentiate between live and dead cells. The cell growth study was replicated twice with an error of <20 % in most cases. The figure also shows the errors in terms of standard deviation of the results

the collagen scaffolds were incubated at 37 °C for 2 h before adding additional medium. All seeding plates were maintained at 37 °C in the humidified incubator and the medium was changed every 2 days.

After 1, 2 and 3 weeks post-seeding, the culture medium was removed and both the control and wells containing scaffold specimens were washed out with PBS solution to discard any remaining medium. The scaffolds were then removed for SEM analysis. Trypsin/EDTA solution was used to detach cells followed by re-suspending in the supplemented DMEM. The mixture solution was centrifuged for 5 min followed by re-suspending in DMEM for cell count using a haemocytometer.

Glucose diffusivity measurement

A diffusion cell was constructed to measure the glucose diffusivity within the seeded scaffolds in cell culture medium (CCM). The design and the operation principle of the diffusion cell have been described in detail (Suhaimi et al. 2015). Briefly, the cell consisted of two acrylic chambers, namely, donor and receptor chambers. Both chambers held equal volumes, 41 ml, with an internal geometry of length 20 mm × ht 45 mm × width 45 mm. The seeded scaffold was fixed in between the chambers (Fig. 2). The donor chamber was filled with 8 mg glucose/ml dissolved in CCM while the receptor chamber contained just CCM. Glucose was dissolved without further purification in a

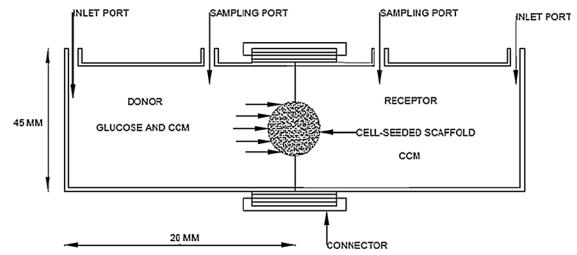


Fig. 2 Schematic drawing of a diffusion cell to measure the glucose diffusivity across the seeded scaffolds saturated in cell culture medium at 37 °C (dimensions of the cell are shown in the figure)

beaker containing CCM before the start of the diffusion experiment). Both solutions of CCM and glucose containing CCM were placed held at 37 °C for 1 h to equilibrate the temperature. The whole apparatus was held at 37 °C.

Changes in glucose concentration were measured using a glucose analyser (YSI 2300 Stat Plus, YSI UK Ltd, Hampshire, UK) (Suhaimi et al. 2015). Samples were taken simultaneously from both chambers using a plastic syringe. The samples were then placed inside a glass cuvette where 25 µl were aspirated by the sipper of the glucose analyser. After the measurements were recorded, the samples were poured back into the diffusion cell to keep the volume constant. The measurements were taken at hourly intervals until equilibrium was achieved.

The diffusion study was conducted at 1, 2 and 3 weeks post-seeding for both PLLA and collagen scaffolds and at 1 and 2 weeks post-seeding for the PCL scaffold. After 1, 2 and 3 weeks post-seeding, scaffold specimens were removed from the well plate and placed in between the chambers of the diffusion cell for diffusion experiments.

The corresponding diffusivities were calculated according to Fick’s first law which was modified to include the effective diffusivity by Gutenwik et al. (2004) defined by:

$$J = -D_e \frac{\partial C}{\partial z} \tag{1}$$

Assuming that there was no change in volume in the diffusion cell, Eq. (1) as translated into Eq. (2) as given below:

$$V_d \frac{\partial C_d}{\partial t} = -D_e A \frac{C_d - C_r}{l} \tag{2}$$

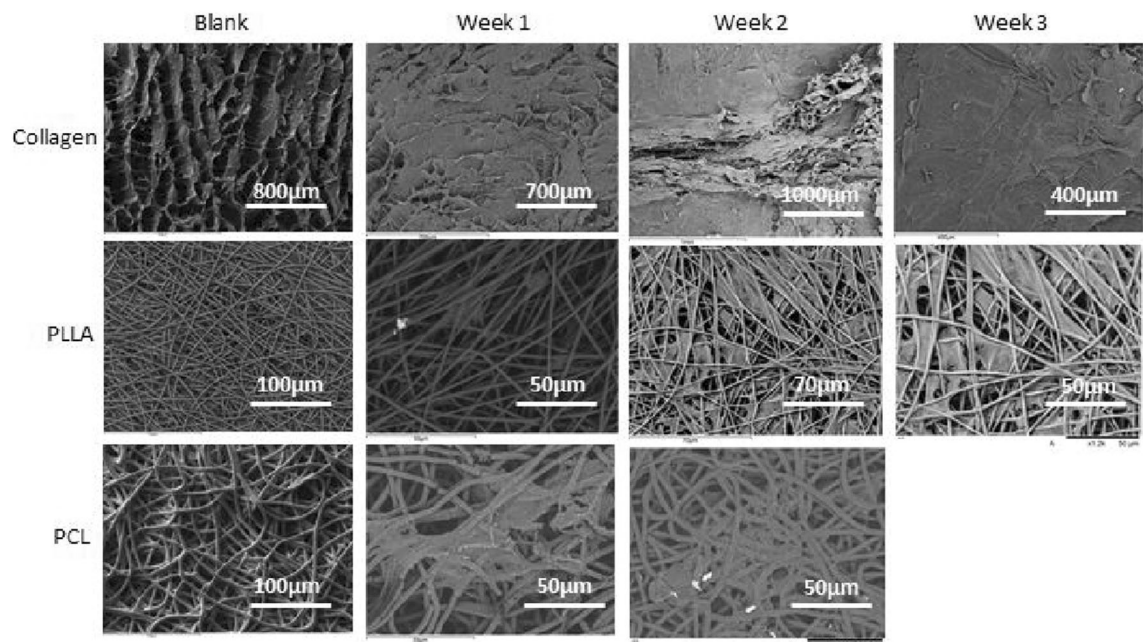


Fig. 3 SEM micrographs showing morphological changes on the surface of collagen, PLLA and PCL scaffolds from no cells attached (blank scaffold) to cells cultured on week 1, week 2 and week 3

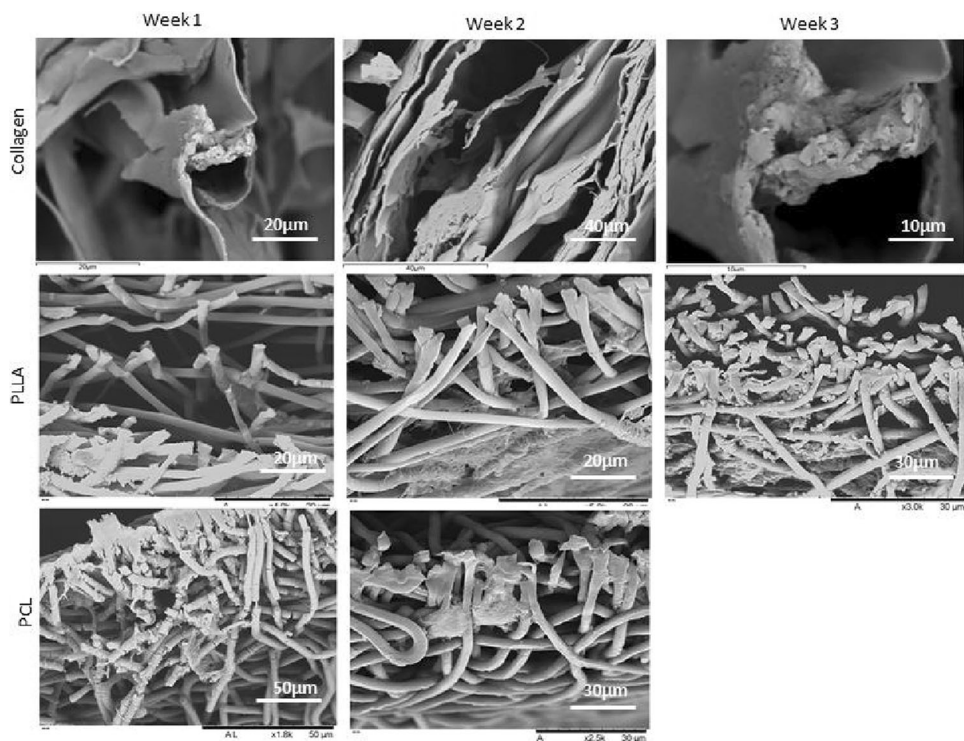


Fig. 4 SEM images showing the cross-sectional cell distribution in collagen, PLLA and PCL scaffolds where cells have migrated into on the time points of week 1, week 2 and week 3 of culture period

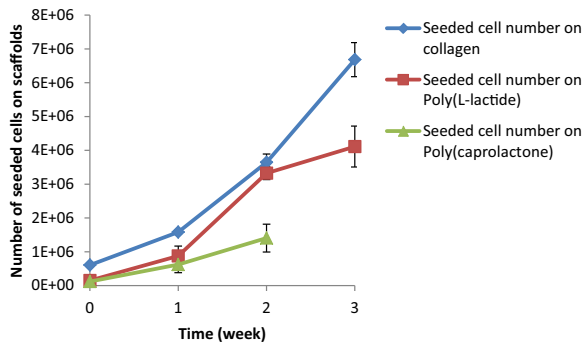


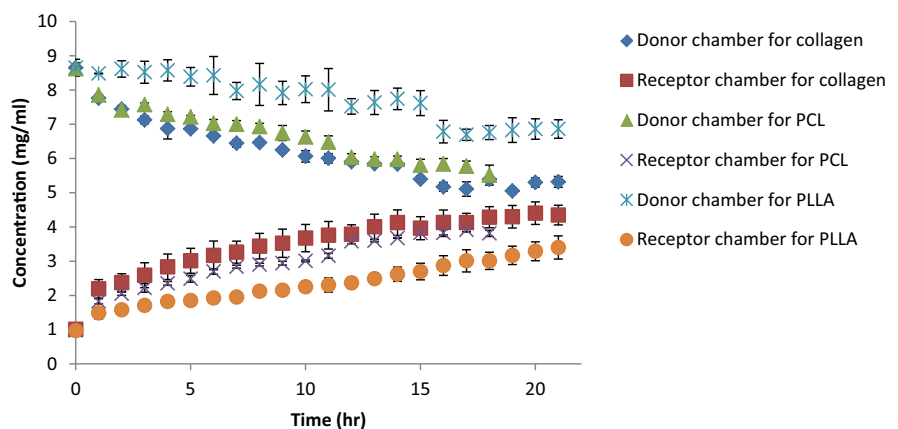
Fig. 5 The approximate number of cells grown on collagen and PLLA scaffolds in week 1, 2 and 3 and that of PCL scaffold in week 1 and 2 of culture periods. The difference between cell number contained inside the control well and cell number contained inside the wells that initially were present with scaffold specimens, represents the number of cells that have grown on the scaffolds. All data were replicated twice with an error of <15 % in most cases. ^{Collagen} Data from 21-day cell growth were used for calculation of number of cells grown in collagen on week 1, 2 and 3. ^{Poly(L-lactide)} Data from 21-day cell growth were used for calculation of number of cells grown in poly(L-lactide) on week 1, 2 and 3. ^{Poly(caprolactone)} Data from 14-day cell growth were used for calculation of number of cells grown in poly(caprolactone) on week 1 and 2

where l = scaffold thickness, A = area of the scaffold, D_e = effective diffusivity of glucose in the seeded scaffold and V_d = donor volume.

The effective diffusivity was determined by fitting the experimental data into Eq. (2) as described in Suhaimi et al. (2015).

All errors in the results were calculated in terms of standard deviations. No other statistical calculation was made. All experiments were repeated twice unless

Fig. 6 Diffusion cell experiments with 8 mg glucose ml^{-1} for cultured collagen, PLLA and PCL scaffolds saturated in cell culture medium at 37 °C. All diffusion experiments were replicated twice with an error of <20 % in most cases



there is significant dispersion in the results in which case the experiments have repeated at least three times.

Results and discussion

As mentioned earlier, we carried out a number of experiments for characterising the materials in our diffusion studies. The thickness, pore size, porosity, minimum, mean and maximum pore size of PLLA are 50, 12–18 μm , 80 %, 4.04, 13.67 ± 4.25 and 25.87 μm , respectively, while that of PCL were 50, 20–30 μm , 80 %, 5.8, 21.69 ± 6.85 and 44.84 μm , respectively. Collagen’s thickness, pore size, porosity, minimum, mean and maximum pore size were 1500, 80 μm , 72 %, 12.55, 75.15 ± 5.21 and 175.18 μm , respectively.

Cell proliferation on scaffolds

To confirm the morphological changes of the cell-seeded scaffolds at various cell culture time intervals, the materials were viewed for surface morphology and cross-sections using SEM. Figure 3 illustrates some typical micrographs of osteoblasts seeded on the surface of collagen, PLLA and PCL scaffolds after 1, 2 and 3 weeks of culture as well as the blank scaffolds (no cells). A clear comparison is depicted on the morphological change between blank scaffolds and seeded scaffolds. The cells gradually covered the surface and almost filled all of the pores by week 3. Figure 4 illustrates the cross-sectional view of the fibres of the scaffold where cells have migrated. More

cells were attached on the surface rather than in between the fibres of the scaffold. Figure 5 presents the number of seeded cells in all scaffolds at 1, 2 and 3 weeks of culture which shows similar pattern as the cell growth kinetics curve (Fig. 1).

Glucose diffusion analysis

Typical curves for the temporal change in glucose concentration for both donor and receptor chambers are shown in Fig. 6 which depict the changes in the two chambers during the diffusion experiment for collagen, PCL and PLLA scaffolds. The diffusion coefficients of the cultured scaffolds were calculated by fitting the experimental data into Eq. (2) and the diffusivity values are listed in Table 1. Table 1 also shows the percentage difference between scaffolds with no cells and cultured scaffolds. Tissue engineering scaffolds should be biodegradable for successful tissue formation in vivo; however, PCL, PLLA and collagen scaffolds were stable for culturing periods of up to three weeks. This shows that they have a long time of degradation in comparison to the culturing periods and diffusion experiments and hence, we define that any biodegradation has no effect on the diffusivity.

There was an obvious decrease in the values of effective diffusivity between scaffolds with no cells attached (blank) and cultured scaffolds on week 1. However, we did not observe significant difference in the effective diffusion coefficients of the cultured scaffolds between weeks 1, 2 and 3 which is likely due to a slower proliferation rate of osteoblast cells. To further quantify the relationship between the effective diffusion coefficient and number of cells grown on and inside the scaffold, a graph of the effective diffusivity against cell number was plotted (Fig. 7) for the PLLA scaffold. This figure shows a decrease in the effective diffusion coefficient values as cell number increases. Both collagen and PCL scaffolds followed similar trends and are not shown in the figure.

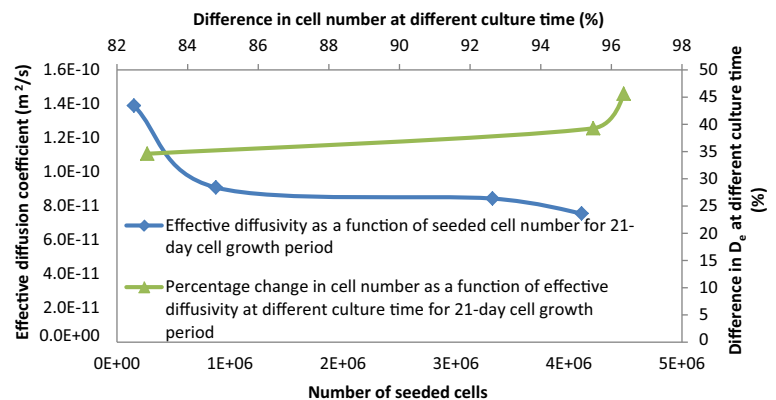
Diffusion is generally defined by a random motion of molecules from a higher concentration to a lower concentration. In this case, glucose molecules have diffused from the donor chamber into the receptor chamber through the connected pores of the scaffold. As the cells gradually cover the surface and almost all of the pores of the scaffold starting from week 1 to week 3, the effective diffusion coefficient of glucose

Table 1 Effective diffusion coefficients with standard deviations for glucose across blank and cultured scaffolds saturated in cell culture medium

Scaffold	Pore size (μm)	Effective diffusion coefficient (m^2/s)			Difference between values calculated from scaffolds with no cells and scaffolds with cells grown on week 1 (%)	
		Effective diffusion coefficient of scaffolds with no cells (Suhaimi et al. 2015)	Effective diffusion coefficient of scaffolds with cells grown on week 1	Effective diffusion coefficient of scaffolds with cells grown on week 2		
Collagen	80	$3.71 \pm 2.78 \times 10^{-9}$	$3.23 \pm 0.16 \times 10^{-9}$	$3.22 \pm 0.16 \times 10^{-9}$	$3.07 \pm 0.11 \times 10^{-9}$	12.9
Poly (L-lactide) (PLLA)	12–18	$1.39 \pm 0.28 \times 10^{-10}$	$9.09 \pm 0.67 \times 10^{-11}$	$8.44 \pm 0.17 \times 10^{-11}$	$7.56 \pm 0.46 \times 10^{-11}$	34.6
Poly(caprolactone) (PCL)	20–30	$1.78 \pm 0.5 \times 10^{-10}$	$1.32 \pm 0.1 \times 10^{-10}$	$1.17 \pm 0.04 \times 10^{-10}$	NA	25.8

The table also shows the errors in terms of standard deviation of the data

Fig. 7 The relationship between effective diffusivity and seeded cell number for PLLA scaffold. The percentage difference for both cell number and effective diffusivity at different culture time were calculated at the time points of 1, 2 and 3 weeks of culture time with reference to initial time (at 0 week)



seems to decrease monotonically with the cell number which is attributed to the change in the pore volume available for diffusion.

Conclusion

A diffusion cell has been constructed to carry out glucose diffusion experiments through cell-seeded scaffolds saturated in cell culture medium at 37 °C. Cell growth changed the morphological structure of the scaffold which then affected the effective diffusion coefficient of glucose. The pore volume for glucose diffusion was reduced and increasing cell mass grown on and inside the scaffold decreased the mobility of glucose. Further investigation on cell proliferation rate by DNA quantification is necessary to understand the relationship between effective diffusion coefficient and culture time. Observing the cell specific glucose consumption rates from one tissue construct to another may also be useful as the consumption rates may vary.

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