Study on Diffusion Coefficient of Fluorophores in 3D Hydrogel with Cationic Charge using Microchip

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1. Introduction

Hydrogels, also well known as aquagels, are composed of a three-dimensional network of hydrophilic polymer chains. Hydrogels can be used to deliver soluble or immobilized signals to the cells, act as support structures for cell growth and function, and provide space filling for the future tissue ingrowth. It is highly absorbent and therefore can swell or shrink by absorbing or emitting water [1–2]. Diffusivity in hydrogels is of significant importance in mimicking the actual biological environment. Besides molecular weight or size, diffusivity can be affected greatly by the polarity or electric charge density of the bio molecules and hydrogels. In this study, the diffusion coefficients of charged molecules are measured in a cationic charged 3D hydrogel by using microchips in order to study the interaction effect of charge conditions of hydrogels.

2. Experimental Methods

A poly-DADMAC solution was synthesized by using 4.8 mL of poly(acrylamide-co-diallyl-dimethyl-ammoniumchloride) solution (Sigma®), 0.05 g of N,N′-Methylenebis (acrylamide; Sigma®) that acted as a cross-linker, and 0.05 g of 2-Hydroxy -4’-(2-hydroxyethoxy)-2-methylpropiophenone (Sig ma®) that acted as a photoinitiator. The synthesized poly-DADMAC solution was injected into the microchannel and cured by using ultraviolet light (UV). Omnicure® S2000 was used as an ultraviolet light source and poly-DADMAC solution was cured for 10 s using 20 W of power.

3. Long-term Diffusivity Experiment

The Poly-DADMAC is loaded into the channel through the use of the capillary force. The photoinitiator added to the Poly-DADMAC allows it to be cured by UV irradiation at 10 Watts for 10 seconds. After UV curing, the channel will appear clogged with Poly-DADMAC, but the Poly-DADMAC has a porous structure through which the fluorophores can diffuse through. The 20 μL of each of the four fluorophores are loaded onto the inlet of the PDMS channel. In order to maintain constant density of the materials at the inlet, a thermohygrostat was used. The materials were added to the inlet once every 24 hours to prevent dehydration, and the diffusion of the materials towards the outlet was observed. The fluorescence intensity of the microchannel at 2.5 mm intervals was measured every 2 days from day 1 by taking a photo using the microscope and the diffusion coefficient was calculated from the fluorescence intensity.

Table 1. Diffusion coefficients in poly-DADMAC measured by the long-term experiment

	Diffusivity (μm^2/s)
Rhodamine 6G	4.629
Rhodamine-BSA	1.323
FITC	5.622
FITC-BSA	5.291

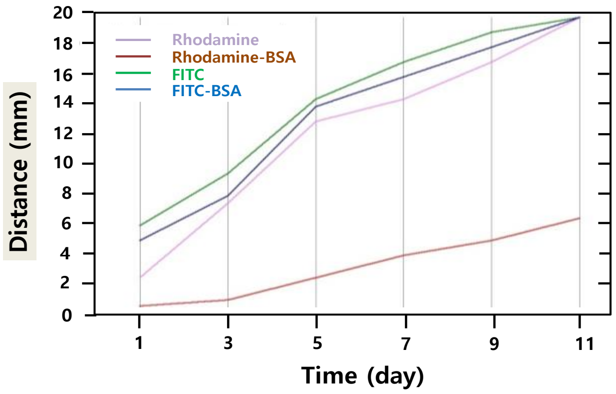

Fig. 1. Diffusion lengths along the microchannel for each measurement. Rhodamine-BSA was the slowest at diffusing and FITC was the fastest.

4. Results and Discussion

a long-term diffusivity test as shown in Table 1 was conducted and the results were compared to those for FRAP. The diffusion coefficients of rhodamine 6G, rhodamine-BSA, FITC, and FITC-BSA when measured by the long-term diffusivity test were 4.629 μm^2/s, 1.323 μm^2/s, 5.622 μm^2/s, and 5.291 μm^2/s, respectively. Fig. 1 shows the diffusion lengths of each fluorophore in the poly-DADMAC cured microchannel. Th e diffusion lengths of rhodamine 6G, FITC and FITC-BSA were same on day 11 because all of the molecules diffused to the end of the microchannel.

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