Study Of Diffusion In Complex Media
By
Fluorescence Correlation Spectroscopy (FCS)

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Outline

- Research overview
- Background
  - Why Hydrogel
  - FCS
- Using FCS in polymer
  1. Solutions
  2. Networks
Research overview

Big picture is:

• Building biomaterial based in-vitro models as platforms for drug screening
• Study disease progression

Specific goal:

• Investigating transport and interactions in complex environments
Hydrogels

- Encapsulate cells
- Absorb a large volume of liquid for nutrient and waste transport.

Role of the tumor microenvironment on drug resistance in solid tumors

- ECM properties emulated by biomaterial scaffolds
- Drug penetration limitations imposed by biomaterial scaffolds and studied via FCS.

Image: www.datlof.com

Image: Minchinton et. al.
In injectable drug delivery to the injured nerve, hydrogels can

- Localize drug release
- Modulate drug release

Fluorescence Correlation Spectroscopy

Image: http://laser.ceb.cam.ac.uk
Diffusion measurements of FCS

- Measures and correlates fluctuations in fluorescence intensity
- Probes molecular diffusion and interaction
- Very small detection volume and concentration

Schematic diagram of FCS. (A) Schematic of observation area of FCS (confocal volume). (B) Obtained fluctuation of fluorescence intensity. (C) Autocorrelation functions (ACFs).

Image: http://www.intechopen.com
Function

- Laser beam provides exciting radiation
- Dichroic mirror pass the light to the objective
- Beam focused on the sample
- Fluorescent light collected by objective

Schematical drawing of an FCS setup

Image: Fluorescence Correlation Spectroscopy an Introduction to its Concepts and Applications /book
FCS data analysis

1) For freely diffusing, mono-disperse, and uniformly bright fluorescent particles:

\[ G(\tau) = 1 + \frac{1}{N} \left( \frac{1}{1 + \frac{\tau}{\tau_d}} \right) \left( 1 + p \frac{\tau}{\tau_d} \right)^{0.5} \]

- \( N \): number of particles in fluorescence volume;
- \( p = \left( \frac{r_o}{z_o} \right)^2 \) : instrumental constant.

\[ \frac{\tau_0}{\tau_d} = \frac{D_e}{D_0} \]

2) For two independent species:

\[ G(\tau) = 1 + m_1 \left( \frac{1}{1 + \frac{\tau}{\tau_1}} \right) \left( 1 + p \frac{\tau}{\tau_1} \right)^{1/2} + m_2 \left( \frac{1}{1 + \frac{\tau}{\tau_2}} \right) \left( 1 + p \frac{\tau}{\tau_2} \right)^{1/2} \]

Where
- \( \tau_1 \) and \( \tau_2 \) – respective characteristic diffusion times
- \( m_1 \) and \( m_2 \) - respective average number.
What is a Polymer?

- Polymers are made up of many many monomers
- Long chains (and sometimes more complicated structures, too)
Diffusion in complex media

Polymer Solutions

Ficoll
PEG
Dextran
Atto488
Rhodamine 6G
BSA

Polymer Networks

PEG-DA
Alginate
Polymer Solutions (Poly ethylene glycol)

- Non-toxic
- Hydrophilic (aqueous-soluble)
- Odorless
- Neutral
- Biodegradable
- Molecular Weight: 3365 g/mole
- Study diffusivity of R6G and Atto488

![PEG structure]

![Rhodamine 6G](image1)

![Atto488 structure](image2)
Preparing solutions

The perfusion chamber is sealed to avoid evaporation.

Perfusion chambers

$C_{R6G}$

PEG solution containing 10 nM of fluorophore

Image: www.stratech.co.uk
Results-PEG Solutions

- Dilute regime
- R6G and Atto488 interact with PEG solutions

**PEG-R6G**

**PEG-Atto488**

<table>
<thead>
<tr>
<th>PEG CONC. (%)</th>
<th>TauO/TauD</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>1</td>
<td>0.085</td>
</tr>
<tr>
<td>0.10%</td>
<td>0.67</td>
<td>0.0582</td>
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<tr>
<td>0.50%</td>
<td>0.66</td>
<td>0.0542</td>
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<tr>
<td>1%</td>
<td>0.64</td>
<td>0.0289</td>
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</table>

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<thead>
<tr>
<th>PEG CONC. (%)</th>
<th>TauO/TauD</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0.217</td>
</tr>
<tr>
<td>0.1</td>
<td>0.597</td>
<td>0.0486</td>
</tr>
<tr>
<td>0.5</td>
<td>0.533</td>
<td>0.0381</td>
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<tr>
<td>1</td>
<td>0.438</td>
<td>0.0334</td>
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</table>

Figure: For all experiments Rhodamin6G CONC. is 10nM
There is interaction between PEG and dyes but BSA shows less interaction with PEG.
Designing experiments to find types of interactions between PEG and dyes

- pH:3
- pH:10
- High salt solutions

There is no significant change for different conditions!
Diffusion in complex media

Polymer Solutions
- Ficoll
- PEG
- Dextran

Polymer Networks
- PEG-DA
- Alginate
- Rhodamin 6G
Polymer Networks

0.25% and 1% gels, Collagen CONC. 0.5 mg/ml

- Alginate
- Alginate-RGD
- Alginate-Collagen
Large network lattice
To design a complex environment, in addition to pure Alginate we added:
1. RGD which is a peptide
2. Collagen which is a protein
Preparing hydrogels

- 0.25% AND 1% Alginate
- Prepared in media without phenol red
- 1-2 drops Calcium Chloride for crosslinking
- Gel is swollen for 24 h to reach thermodynamic equilibrium.
- The chamber is sealed to avoid evaporation.
Diffusion of R6G does not show significant change for 3 different conditions.

Figure 1: (a) Normalized autocorrelation function curve; results shows same changes in autocorrelation curve for different concentration of Alginate gel (b) Residual for 0.25% Alginate (c) Residual for 1% Alginate (d) Residual for R6G in clear media.
## Results - Alginate network

<table>
<thead>
<tr>
<th>Alginate Concentration (%)</th>
<th>Collagen Concentration (mg/ml)</th>
<th>$\tau_D \pm SD$ ($\mu$s)</th>
<th>$\tau_O/\tau_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25%</td>
<td>0.5</td>
<td>90.289±6.716</td>
<td>0.669</td>
</tr>
<tr>
<td>1%</td>
<td>0.5</td>
<td>85.624±4.705</td>
<td>0.704</td>
</tr>
<tr>
<td>0.25%</td>
<td></td>
<td>89.175±4.256</td>
<td>0.676</td>
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<tr>
<td>1%</td>
<td></td>
<td>86.674±7.118</td>
<td>0.698</td>
</tr>
</tbody>
</table>
Tolerance of human glioblastoma cells to the tested neurotoxins

Stiffer matrix (1%) has more tolerance to toxins in compare with softer condition (0.25%)
QUESTIONS?