Protein Adsorption to Biomaterials

Radioactive Labelling Analysis & Atomic Force Microscopy

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Background

Biomaterials
Materials that interact with a biological system
Materials that are part of implants, drug delivery systems, biomedical equipment, disposable devices, biosensors etc.

Protein adsorption
First step in a cascade of events that eventually can result in non-wanted responses
Influence on functionality of designed systems

Controlling protein adsorption
Manipulating with response
Surface characteristics

Blood protein adsorption onto polymer materials
Albumin (67 kDa), IgG (150 kDa) & fibrinogen (340 kDa)
Radioactive multi-label system & AFM
Background

PET (polyethylene terephthalate)

DEGVE (diethylene glycol vinyl ether)

Plasma polymerisation

- Argon pre-treatment
- Plasma copolymerisation
- Working pressure 5-50 Pa
- 2 phase 50 Hz AC power supply
- 100 Hz pulsed plasma current
- Energy \( \sim 1-5 \text{ Watt} \)

\[ \begin{align*}
\text{PET:} & \quad \begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{C} \\
\end{array} \\
\text{DEGVE:} & \quad \begin{array}{c}
\text{H}_2\text{C} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{H} \\
\end{array}
\]

Radioactive Labelling

Quantitative Protein Adsorption Analysis

Solution with
\(^{125}\text{I}-\text{albumin}\)
\(^{131}\text{I}-\text{IgG}\)
\(^{123}\text{I}-\text{fibrinogen}\)

Rinsing Procedure

Gamma counting

Quantitative technique for Competitive Adsorption from Complex Solutions

Function of time, concentration, surface characteristics etc.

\[ T = t_1 \quad T = t_2 \]

Intensity

keV

Adsorption, ng/cm\(^2\)

Time, min

0,01 0,1 1 10

0 50 100 150 200 250 300
Competitive Protein Adsorption

Alb & IgG adsorption onto PET & DEGVE (1 h & 24 h)

PET – decrease with increased human serum %

DEGVE – increase with human serum %

M. Holmberg et al., Langmuir 26 (2010), 938
Alb & IgG adsorption onto PET

Single protein adsorption versus Competitive protein adsorption onto PET

M. Holmberg et al., Langmuir 25 (2009), 2081
Alb & IgG adsorption onto PET

Protein adsorbed, ng/cm²

Adsorption time, minutes

- Albumin 0.1 mg/ml
- IgG 0.03 mg/ml
- Albumin from 0.25 % Human Serum
- IgG from 0.25 % Human Serum

Aggregation? Multilayer?

Single protein adsorption

Competitive protein adsorption

Influence from presence of other proteins

Specific interaction between protein and surface
Adsorption of Alb & IgG onto PET & DEGVE

10 mg/ml Alb & 3 mg/ml IgG (25 % human serum)

Different adsorption times (1 min versus 1 h)

Single protein adsorption

Competitive protein adsorption

Sequential protein adsorption
Alb & IgG adsorption onto PET

**Protein adsorption, ng/cm²**

- **Albumin**
- **IgG**

**Single Protein & Competitive Protein Adsorption**

- 1 min Alb
- 1 min IgG
- 1 min (Alb+IgG)
- 1 h Alb
- 1 h IgG
- 1 h (Alb+IgG)

**Sequential Adsorption**

- 1 min Alb + 1 h IgG
- 1 min IgG + 1 h Alb

*M. Holmberg et al., Langmuir 25 (2009), 2081*
Alb & IgG adsorption onto DEGVE

![Graph showing protein adsorption](image)

- **Protein Adsorption**
  - Albumin
  - IgG

**Sequential adsorption**

- Single Protein & Competitive Protein Adsorption

**Protein adsorption, ng/cm²**

- 1 min Alb
- 1 min IgG
- 1 min (Alb+IgG)
- 1 h Alb
- 1 h IgG
- 1 h (Alb+IgG)
- 1 min Alb + 1 h IgG
- 1 min IgG + 1 h Alb

*M. Holmberg et al., Langmuir 25 (2009), 2081*
Conclusions – IgG adsorption

Specific protein-surface interaction – *surface induced protein aggregation*

Influence from presence of other proteins – *competition and blocking of surfaces*

Non-fouling characteristics of DEGVE – *lower adsorption*
Alb & IgG adsorption onto PET and DEGVE

Protein adsorption in ng/cm²

PET

- 1 min Alb
- 1 min IgG
- 1 min (Alb+IgG)
- 1 h Alb
- 1 h IgG
- 1 h (Alb+IgG)
- 1 min Alb + 1 h IgG
- 1 min IgG + 1 h Alb

DEGVE

- 1 min Alb
- 1 min IgG
- 1 min (Alb+IgG)
- 1 h Alb
- 1 h IgG
- 1 h (Alb+IgG)
- 1 min Alb + 1 h IgG
- 1 min IgG + 1 h Alb
Atomic Force Microscopy

Tapping Mode in air, PET

Sq = 0.995 nm

Tapping Mode in air, PET + 3 mg/ml IgG

Sq = 0.980 nm

Tapping Mode, PET + 10 mg/ml Alb

Sq = 0.874 nm
Atomic Force Microscopy

Closed system using a liquid cell and o-ring

PET + 10 mg/ml albumin, Imaging in Tapping Mode, 5x5 µm

Scratching in Contact Mode, 500x500 nm

Strength in force

∼15 nm
Summary

Radioactive Multi-Labelling - quantitative analysis of competitive protein adsorption from complex solutions

Evaluating Biomaterials
- Characteristics of protein and surface
- Difference in adsorption patterns
- Specific interaction between protein and surface
- Presence of other proteins

Understanding the protein-surface interaction
Design of biomaterials and devices
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