Correlating the conformation of adsorbed fibronectin with cell growth behaviors on salt etched polyelectrolyte multilayers

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Protein adsorption plays a major role in determining the biocompatibility of materials. The first stage of implant integration is the adhesion of protein followed by cell attachment[1]. It was found that the protein conformation and activity can affect the cell behavior such as adhesion, migration, proliferation and differentiation[2,3] . Layer by layer assembly technique has been widely used in biomedical fileds, especially for surface modification[4]. Here we describe the adsorption characteristics of fibronectin on salt etched PEI(PSS/PDDA)$_7$ multilayers: bovine fibronectin (Fn), the most important ECM protein involved in cell adhesion、migration and cell differentiation. Then correlating the conformation of adsorbed Fn with cell growth behaviors on salt treated polyelectrolyte multilayers.

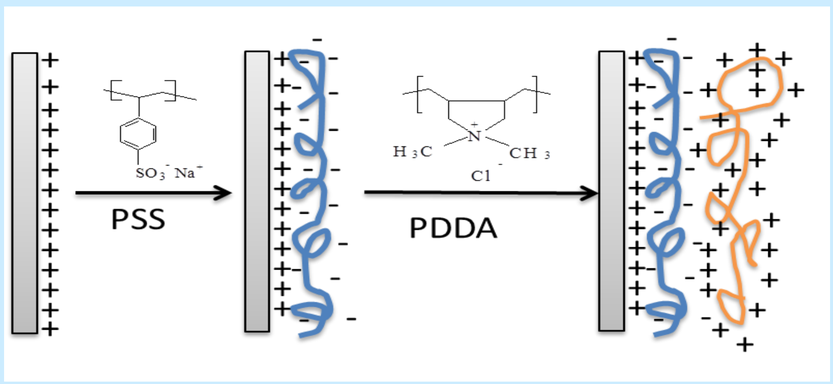

Fig. 1 Schematic diagram of multilayers assembly of PEI(PSS/PDDA)$_7$.

Post-treatment of the multilayers with NaCl

The (PSS/PDADMAC)$_7$ multilayers were incubated in NaCl solutions of different concentrations at room temperature for 2 h and were then rinsed with water and dried under a smooth stream of N_2. The structure of multilayers were changed, as indicted in **Fig. 2**.

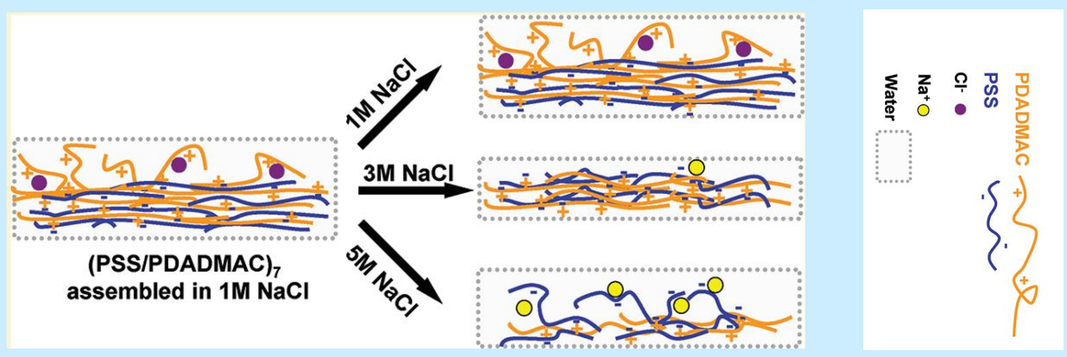

Fig. 2 Schematic illustrations showing the typical physicochemical properties of the (PSS/PDDA) $_7$ multilayers treated with different concentrations of NaCl solutions.

Protein adsorption on different concentration of NaCl treatment multilayers

We quantified the amount of Fn adsorption from 10% serum on 1M、3M and 5M NaCl treated PEMs using fluorescence spectroscopy (see **Table 1**). 1M NaCl treated PEMs exhibited significantly ($p < 0.05$) higher FN adsorption than others, This was because proteins are drawn into the multilayer when the surface is of opposite charge, whereas they remain on the surface when it is like-charged.

Table 1. Density of protein adsorbed on PEMs films by fluorescence spectroscopy.

	Fn in PBS (20μg/ml) Density (μg/cm^2)	Fn in 10% FBS (20μg/ml) Density (μg/cm^2)
Multilayer-1M	**0.51±0.04**	**0.43±0.06**
Multilayer-3M	**0.28±0.03**	**0.28±0.02**
Multilayer-5M	**0.26±0.09**	**0.22±0.04**

The secondary structural content of fibronectin adsorbed on NaCl treated PEI(PSS/PDDA)$_7$ multilayers was detected by ATR-FTIR is presented in **Table 2**.

Table 2. Secondary structural content of Fn in solution (native) and adsorbed on salt etched PEMs by ATR-FTIR.

Fn	β-sheet(%)	Random coil (%)	β-turn(%)
Native	**34.9-38.8**	**31.5-33.8**	**29.8-31.7**
Multilayer-1 M	**23.67**	**32.98**	**43.35**
Multilayer-3 M	**27.95**	**46.17**	**25.88**
Multilayer-5 M	**23.34**	**40.39**	**36.27**

The RGD activity of Fn adsorbed on multilayers were detected by ELLISA Kit(**Fig. 3**).

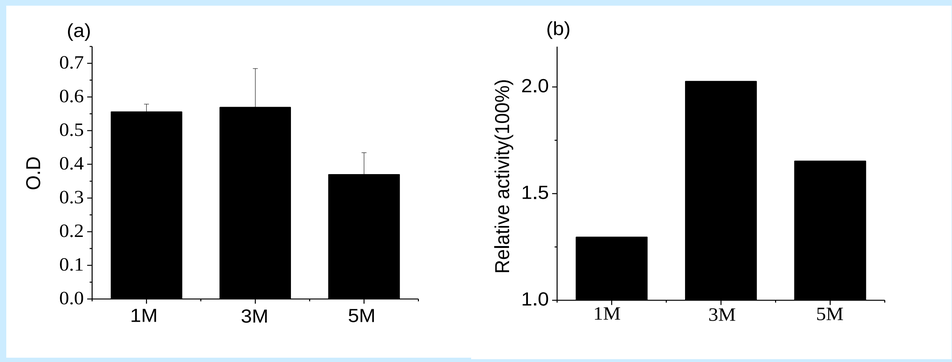

Fig. 3 (a) the RGD activity of Fn adsorbed on different concentration of NaCl treated multilayers. (b) the relative activity of Fn adsorbed on different concentration of NaCl treated multilayers (Relative activity = O.D. detected by ELISA / protein density detected by fluorescence spectroscopy)

From **Table 2**, found that the random coil content of Fn adsorbed on multilayer-3M was highest, then the multilayer-5M, the lowest was the multilayer-1M. In **Fig. 3**, we could see that the relative activity of RGD of Fn adsorbed on multilayer-3M was the highest, then the multilayer-5M, at last, was the multilayer-1M, which correlated well with the random coil content of Fn adsorbed on different multilayers.

HSMC adhesion and migration on multilayers

To investigate whether fibronectin plays a role in affecting the adhesion of Human vascular smooth muscle cells (VSMC) on salt etching PEMs, VSMC cells (25k/well, 24 well culture plate) were seeded on the salt etched PEMs in 10% serum and 10% Fn depletion serum for 24 h (**Fig. 4**).

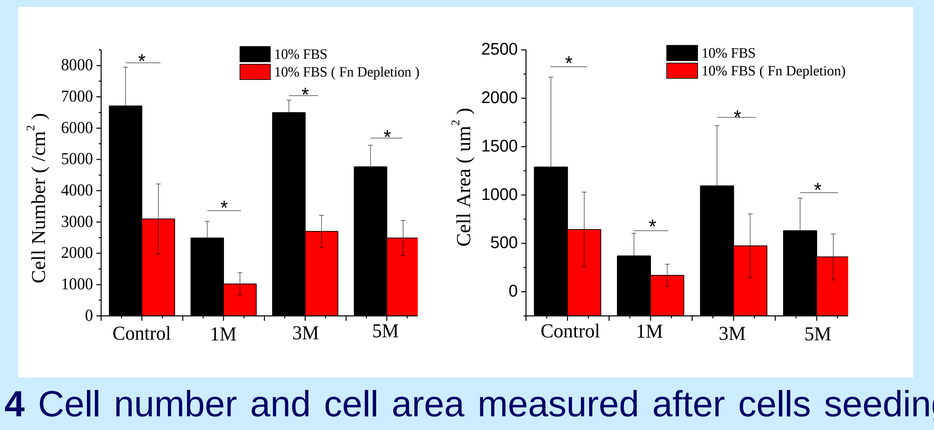

Fig. 4 Cell number and cell area measured after cells seeding on multilayers 24 h with 10% serum and Fn depletion serum. (25K/well), * represents at the 0.05 level, they are significantly different.

From **Fig. 4**, we could see that cell numbers and cell spreading areas are significantly lower in Fn depletion serum than normal serum, especially in 3M NaCl etched PEMs. So we investigated that Fn played an important role in mediating VSMC adhesion on salt etched PEMs.

Acknowledgement

Financial supports by the Major State Basic Research Program of China (2011CB606203) and the Natural Science Foundation of China (20934003)

References

[1]Bengt Kasemo, *Surface Science* ,2002, **500**, 656-667.
[2]B.Sivaraman,RA.Latour,*Biomaterials,*2011,**32**,5365-5370.
[3]BenjaminGeiger,*NatureReview*,*MolecularCellBiology*,2009,**10**,21-3.
[4]Tang, Z.Y. *Advanced Materials*, 2006,**18**, 3203-3224.
[5]Lulu Han, Changyou Gao,Langmuir,2012,**28**,193-199.