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Modulating the behaviors of C3A cells via surface charges of polyelectrolyte multilayers

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ABSTRACT

The purpose of this study was to evaluate in vitro how the modulating surface charges of materials influenced the behaviors of hepatocytes. Cells of a human hepatocyte cell line, C3A, which have been used in a clinically tested bioartificial liver, were conducted as cell models. Polyelectrolyte multilayers (PEMs) of poly-L-lysine and alginate biopolymers were fabricated and then the zeta potential was assessed. Protein adsorption study showed that fibrinogen deposition could be modulated via tuning the terminal layer and the surface charges of PEMs. Furthermore, through observing the cellular morphology, viability, functional protein analysis and gene expression, we found that the behavior of C3A cells could be modulated via tuning of surface charges on PEMs, which was different from that via grafting functional groups on PEMs. It suggested that the PEMs with different charges could be used in vitro to manipulate cell behaviors to improve upon the design of tissue engineering.

1. Introduction

Liver tissue engineering is a promising scientific approach that attempts to create functional cellular constructs within a biomaterial support device (Strain & Neuberger, 2002). Up to now, a variety of strategies have been used to provide a scaffold of biomaterials for culturing primary hepatocytes, hepatic stem cells or hepatic cell lines to form liver-like tissues and maintain their differentiated functions (Elkayam, Amitay-shaprut, Dvir-ginzberg, Harel, & Cohen, 2006; Glicklis, Shapiro, Agbaria, Merchuk, & Cohen, 2000; Khalil et al., 2001; Lozoya et al., 2011). The phenotype of cells is generally dependent on the reciprocal and dynamic interactions of cells within their surrounding microenvironment, which includes biochemical and mechanical stimuli defined by neighboring cells and extracellular matrix (Griffith & Swartz, 2006; Kim et al., 2011; Yamada & Cukierman, 2007). It is becoming increasingly clear that the functional tissues in vitro largely depend on the properties of biomaterials. Thus, a key challenge is to optimize the cell–biomaterial interaction, which can further promote cell survival and function.

Biomaterials utilized in liver tissue engineering must exhibit appropriate chemical, mechanical and biological properties to allow cells to efficiently attach, proliferate and function. A large number of studies have highlighted the effects of stimuli on cellular behaviors ranging from adhesion and motility to functions (Ng et al., 2005; Seo et al., 2005). Generally, hepatocytes tend to attach, proliferate and form flat monolayer cultures, but exhibit limited function on rigid materials containing adhesion-promoting ligands. In contrast, hepatocytes exhibit rounded morphology and improved function, but not proliferate on soft materials with fewer adhesion-promoting ligands (Chen, Khetani, Lee, Bhatia, & Van Vliet, 2009; Chen, Ogawa, Kakugo, Osada, & Gong, 2009; Schrader et al., 2011). In addition, by manipulating the charge density on the surface of biomaterials, cellular attachment can also be altered (Chen, Khetani, et al., 2009; Chen, Ogawa, et al., 2009; Nagavasu et al., 2012; Schneider et al., 2004). It is generally believed that protein adsorption is the first event happening on the surface of any materials and that the adsorbed protein layer mediates further biological reactions, such as the cellular attachment (Martins, Merino, Mano, & Alves, 2010; Wilson, Clegg, Leavesley, & Pearcy, 2005; Xie, Zheng, et al., 2010). Therefore, the understanding of the material–protein–cell interaction is a key for the development of tissue engineering.

Polyelectrolyte multilayers (PEMs), formed by the layer-by-layer (LbL) adsorption of positively and negatively charged polyelectrolytes, are promising substrata for understanding the interaction between materials, proteins and cells (Wittmer et al., 2008). The properties (composition, terminal layer, stiffness, charge, thickness, and presence of biofunctional species) of the film can be controlled very precisely by the choice of polymer, solution conditions, cross-linking steps, and number of layers. Up to now, various PEMs have been used for decoupling the effects of stimuli on cellular processes (Berg, Yang, Hammond, & Rubner, 2004; Jessel et al., 2003; Picart et al., 2005; Richert et al., 2002,

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2004). These PEMs are generally composed of the polysaccharides chitosan (CHI) and alginate (ALG), the poly-L-glutamic acid (PGA) and polypetides poly-L-lysine (PLL), and the synthetic polymers poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS). Several studies have investigated the stimuli of PEMs on the phenotype of hepatocytes (Wittmer et al., 2008). Therefore, the PEMs are ideal substrata for studying the effect of surface stimuli on the fate of cells.

In this study, PEMs composed of ALG and PLL were firstly fabricated, and then the surface charges of the films were investigated. The surface charge was determined by zeta potential measurements. Afterwards, bovine fibrinogen was selected for adsorption study. Furthermore, cells of a human hepatocyte cell line, C3A, which have been used in a clinically tested bioartificial liver support system, were conducted as cell models. The effects of surface charges on the adhesion, morphology and phenotypic functions of C3A cells were studied. The results showed that both the protein adsorption and cellular behaviors were influenced by the surface charges, which was different from that via grafting functional groups on PEMs. It suggested that the surface charges might modulate the protein adsorption, which further influenced the behavior of cells.

2. Materials and methods

2.1. Materials

Alginates (ALG) with a low content of guluronic acid were purchased from Qingdao Crystal Salt Bioscience and Technology Corporation (Qingdao, China). Poly-L-lysine (PLL, *M*w 29,000), fibrinogen (Bovine Plasma) was purchased from Sigma. Fibrinogen at 3.0 mg/mL in 0.9% (w/v) NaCl solution was prepared in sterile conditions (by 0.22 μm filtration) before use. Albumin holds a negative net charge for pH = 7.4. The pH of all solutions was adjusted at a pH of 7.4 by using acetic acid and sodium hydroxide solutions. All other chemicals used were of analytical grade reagent or the best available quality and double-distilled water was used throughout the experiment.

2.2. Multilayer films in 24-well plates

Standard 24-well polystyrene plates are employed. Aqueous solutions containing negatively charged ALG and positively charged PLL solutions (1 mg/mL in 0.9% (w/v) NaCl solution) were prepared first. The well substrate was immersed into the PLL solution for 15 min and then rinsed. This process produced a single layer PLL film (L1). The L1 was then immersed into the ALG solution, rinsed and dried. This process produced a 2 layer PLL/ALG film (L2). This process was repeated more times to prepare a multilayer $(PLL/ALG)_n$ or a multilayer $(PLL/ALG)_n$-PLL film.

2.3. Zeta potential measurements

Zeta potential is an important and useful indicator of surface charge. The zeta potentials of membrane were determined using the SurPASS Electrokinetic Analyzer (Anton Paar GmbH, Austria) equipped with a slit-type channel (Xie, Li, et al., 2010). The streaming potential was measured by the Ag/AgCl electrodes. The multilayer films were fabricated within the well of 24-well polystyrene plates. For each measurement, the plates coated with multilayer films were cut down. Then the plates were transferred into the glass cylinder of the measuring cell. Before starting the measurement, the membranes were rinsed with double distilled water to remove NaCl. A background electrolyte of 1 mmol/L KCl solution was used. The zeta potential was obtained from the streaming potential measurements based on the Smoluchowski equation (Xie, Li, et al., 2010). Since the surface conductivity of the membranes cannot be determined directly, the zeta potential obtained from the streaming potential measurement is considered an apparent value.

2.4. Measurement of protein adsorption onto films

Fibrinogen was used for the protein adsorption assay. The adsorption experiments were performed in 10 mmol/L phosphate buffer (KH_2PO_4/Na_2HPO_4) in order to keep a constant pH during the adsorption process. The membranes were immersed in buffer solution containing protein (1.0 mg/mL) at 37 °C for 24 h to achieve adsorption equilibrium. Protein concentration of the centrifuged supernatant was measured by the Bradford method. The protein adsorbed on membranes was calculated using the following equation:

$$q = \frac{(C_i - C_f)V}{m}$$

where C_i and C_f are the initial protein concentration and the protein concentration in the supernatant after adsorption studies, respectively. V is the volume of the solution and m is the surface area of membranes.

2.5. Cell culture and microscopy

Human hepatocarcinoma C3A cells (purchased from the American Type Culture Collection, ATCC) were cultured in MEM medium (Sigma) supplemented with 2.2 g/L sodium bicarbonate, 10% heat-inactivated fetal bovine serum, 100 U/mL streptomycin and 100 U/mL penicillin. They were incubated in a humidified incubator at 37 °C in 5% CO_2 and 95% air atmosphere. Half of the medium was refreshed every day, and the morphology of the entrapped C3A cells was observed under an optical microscopy (Olympus CK40, Tokyo, Japan).

2.6. Cell counting

After detaching the cells from monolayer, cells were assessed using a hemocytometer with trypan blue staining. Measurements were performed on every day. Results were expressed as mean ± SD for three replicates.

2.7. Realtime RT-PCR

Two-step quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed on RNA from cultures according to the manufacturer's instructions (PrimeScript™ RT reagent Kit, SYBR *Premix Ex Taq*™ II, TaKaRa). After cultured for 6 days, total cellular RNA in different groups was extracted with Trizol agent. Total RNA was quantified by UV spectrophotometry. RNA was reverse-transcripted with oligo (dT) primers and then amplified. The thermal profile was 37 °C for 15 min, then 85 °C for 5 s, followed by 40 amplification cycles consisting of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min. Primers were designed using Oligo 6.0 software to span introns and custom made by Operon. All primers were synthesized by Takara. Table 1 shows the design of primers of genes related to functions of hepatocytes.

2.8. Albumin enzyme-linked immuno-sorbent assay

Cell culture supernatants were collected and albumin concentration determined by a human albumin enzyme-linked immunosorbent assay (ELISA) kit per the manufacturer's instructions (Bethyl, Montgomery, TX, USA). The absorbance was determined using a plate reader (Infinite F50, Tecan Co., Switzerland).

Table 1
The design of primers of genes related to functions of hepatocytes.

Target gene	Primer sequence (5′–3′)	Length of product (bp)	GeneBank accession no.
CYP1A1	F – CGGCCCCGGCTCTCT R – CGGAAGGTCTCCAGGATGAA	65	NM_000499
CYP3A4	F – CAGGAGGAAATTGAT-GCAGTTTT R – GTCAAGATACTCCATCTG-TAGCACAGT	78	NM_017460
EPHX1	F – TGAGAACGTGGAGCCTGGTG R – AGTAGATGGCAAAGCCCAGCA	199	NM_000120.3
UGT1A1	F – TGGCTGTTCCCACTTACTGCAC R – AGGGTCCGTCAGCATGACATC	82	AY4355136
GSTA1	F – CAAGTGCCAATGGTTGA-GATTGA R – CAGGTGGACATACGGGCAGA	189	NM_145740.3
NDUFA3	F – CAAGAATGCCTGGGACAAGGA R – GCCTTGTTGATCATGACG-GAGTA	117	NM_004542.3
GCLM	F – CAGCTGTTGACTCACAAT-GATCCA R – TGCGCTTGAATGTCAGGAATG	92	NM_002061.2
GAPDH	F – GGAAAGCTGTGGCGTGAT R – AAGGTGGAAGAATGGGAGTT	308	NM_017008

2.9. Statistical analysis

All experiments were carried out at least three times. All values were expressed as means ± standard deviations. Statistical analyses were performed with Student's *t*-test, with $p < 0.05$ being regarded as statistically significant.

3. Results

3.1. Zeta potential analyses

The charges on the surface of materials are one of important properties mediating further biological activities, such as protein absorption and cellular attachment. Charged films are characterized by their zeta potentials, which are the potentials at the hydrodynamic slipping plane adjacent to the phase boundary. Fig. 1 displays the zeta potential of respective outermost shell measured during the preparation of the $(PLL/ALG)_5$-PLL film. The zeta potential of PLL film was −9.8 mV. The zeta potential of $(PLL/ALG)_n$-PLL film and $(PLL/ALG)_n$ film was positive and negative, respectively. With regard to $(PLL/ALG)_n$-PLL film, there were a large amount of positively charged amine groups and minority of negatively charged carboxylic groups being not sufficiently neutralized. Thus, the zeta potentials of $(PLL/ALG)_n$-PLL film were positive at a neutral pH. Conversely, the zeta potentials of $(PLL/ALG)_n$ film were negative at neutral pH. This oscillation in the zeta potential was attributed to the stepwise immersion in positively charged PLL solution and negatively charged ALG solution, respectively. The alternating reversals of zeta potential are characteristic of the LbL assembly, indicating stepwise adsorption of PLL and ALG onto the substrate.

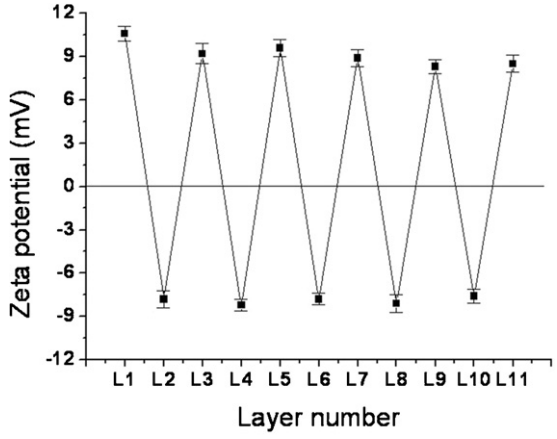

Fig. 1. Zeta potential of respective terminal shell measured during the preparation of $(PLL/ALG)_5$-PLL films.

3.2. Protein adsorption onto PEMs

Protein adsorption onto these PEMs made of different layer number and terminal layer was studied. It seemed that the layer number has nothing to do with the amount of adsorbed fibrinogen. However, the amount of adsorbed fibrinogen on films of PLL terminal layer was significantly higher than that on films of ALG terminal layer. In this study, a comparison between zeta potential (Fig. 1) and protein adsorption (Fig. 2) showed that the positively charged groups on the surface of films increased the amount of adsorbed protein. Whereas, the negatively charged groups on the surface of films decreased the amount of adsorbed protein.

Films of ALG terminal layer had a net negative charge at neutral pH. However, some negatively charged protein still could adsorb onto these PEMs with the ALG terminal layer despite the electrostatic repulsion. The ALG of the outer layer may not cover entirely the surface so that the beneath opposite charge chains might still emerge. The existence of positively charged amino groups on the surface weakened the electric repulsion. Thus, fibrinogen exhibited a small number of adsorption behavior on the PEMs with the ALG terminal layer. The results gave evidence that electrostatic

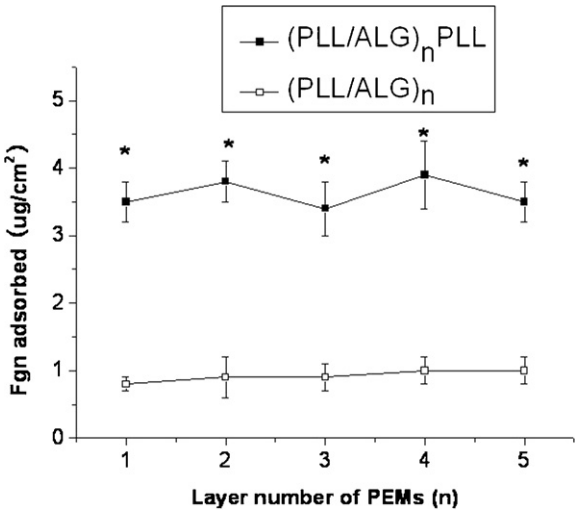

Fig. 2. Protein adsorption onto PEMs films made of different terminal layer at pH 7.4.

interaction was a driving force for the adsorption of proteins on the surface of PEMs.

3.3. Morphology cells

C3A cells cultured on the tissue culture polystyrene (TCP) was considered as the control group. In the beginning of the culture, cells distributed evenly as single cell on the surface of films (Fig. 3). Cells firstly adhered on TCPs and then proliferated as two dimensional (2D) spread. Eventually they became confluent as a monolayer. On PEMs with a terminal layer of PLL, cells adhered on the films and grew to form a confluent monolayer, which was similar to the morphology of cells on TCPs. In contrast, cells on PEMs with a terminal layer of ALG formed three dimensional (3D) multi-aggregates. In addition, the morphology of cells was not affected by the layer number of PEMs. With regard to PEMs with the same terminal layer and different layer number, the morphology of cells was similar to each other.

3.4. Proliferation and viability of C3A cells

The total cell number and cell viability during the culture were shown in Fig. 4A and B, respectively. C3A cells cultured on the TCPs were considered as the control group. The growth of cells cultured on the $(PLL/ALG)_3$-PLL film and $(PLL/ALG)_3$ film was investigated. In the beginning of the culture, the number of cells in each sample was the same to each other. As the culture time went on, the number of cells in the control group (TCPs) increased dramatically and reached a maximum at about day 4. The proliferation of cells on the $(PLL/ALG)_3$-PLL film showed the similar trend with that on TCPs. Compared with the control group, cells cultured on PEMs with a terminal layer of ALG showed a lower proliferation. Cells on $(PLL/ALG)_3$-PLL film grow in 2D culture until confluency, while cells on $(PLL/ALG)_3$ film grow in 3D culture and showed limited proliferation.

The viability of C3A cells in each well of 24-well culture plate was evaluated by using the trypan blue assay after trypsinization. Fig. 4B showed the distinctive viability of the cells in each type of culture. A 90% viability was maintained after 6 days for the monolayer culture on TCPs, while over 87% and 83% viability was maintained for cells cultures on PEMs with terminal layer of PLL and ALG, respectively. There was no significant difference in the viability between the $(PLL/ALG)_3$-PLL film and $(PLL/ALG)_3$ film.

3.5. Gene expression analysis

Through the real-time PCR, Fig. 5 showed the gene expression of C3A cells in different cultures. The expression products of these genes engaged in xenobiotic and synthetic metabolism. In this study, the types of culture systems did not alter the expression the housekeeping gene, GAPDH. Most expression levels of these genes was markedly upregulated in the cultures on the PEMs with a terminal layer of ALG. Real-time PCR also declared that the gene expression was also influenced by the terminal layer of PEMs (PLL vs ALG). Fig. 5 showed the up-regulation of xenobiotic metabolism-associated transcription factors in C3A cells cultured on PEMs with a terminal layer of ALG versus PLL (CYP1A1 6.6-fold, CYP3A4 3.9-fold, EPHX1 4.5-fold, and UGT1A 1.9-fold). In addition, the gene expression of synthesis-associated transcription factors in C3A cells cultured on PEMs with a terminal layer of ALG versus PLL (GSTA1 8.2-fold, NDUFA3 1.5-fold, and GCLM 3.0-fold). These results suggested that the functional gene expression of cells might be susceptible to the terminal layer of PEMs.

3.6. Albumin secretion

The specific rate of albumin secretion by the cells to the external medium, considered as an indicator of hepatocellular synthetic function, was tested by ELISA. The amount of albumin secreted in single cells was determined to assess the synthetic function of cells. As showed in Fig. 6, there was no significant difference in the albumin secretion per cells between the TCPs and PEMs with a terminal layer of PLL. After 6 days' culture, the albumin secretion per cells on the PEMs with a terminal layer of ALG is about 2 fold than that on the PEMs with a terminal layer of PLL. Therefore, the PEMs with a terminal layer of ALG may provide an ideal surface, which is conducive to maintaining the liver-specific function for hepatocytes.

4. Discussion

It is becoming increasingly clear that the cell attachment largely depend on the properties of biomaterial surface. Adhesion of cells to the biomaterials plays an important role in influencing vital aspects of anchorage dependent cell behaviors (Martins et al., 2010). For example, on substrata containing adhesion-promoting ligands, hepatocytes tend to attach, proliferate and form flat monolayer cultures, but exhibit limited function. In contrast, on substrata with few adhesion-promoting ligands, hepatocytes form aggregates and exhibit few proliferation (Chen, Khetani, et al., 2009; Chen, Ogawa, et al., 2009; Picart et al., 2005). Up to now, many studies have investigated the behaviors of hepatocyte on various substrata containing functional groups such as RGD ligands (Picart et al., 2005; Stabenfeldt & Laplaca, 2011). Besides the functional groups, the surface charge on the material surface is another important factor mediating the fates of cells. In this study, we have utilized PEMs to evaluate the effects of surface charges on the adhesion, morphology, and phenotypic functions of hepatocytes, which are considered important for liver tissue engineering.

The behavior of C3A cells is correlated with the charged PEMs, since PLL is positively charged while ALG is negatively charged. On the PEMs absent of RGD-motifs of ECM proteins, the interaction between the PEMs and cells is nonspecific. With regard to this interaction, Richert et al. have reported that pure electrostatic repulsion and attraction forces between cell membranes and the terminal layer of PEMs determined the cellular attachment (Jessel et al., 2003). Besides, increasing evidence has shown that adsorption of proteins on the surface of biomaterials played an important role in mediating the behavior cells. When the biomaterials come into contact with the host environment in vivo or the cells in vitro,

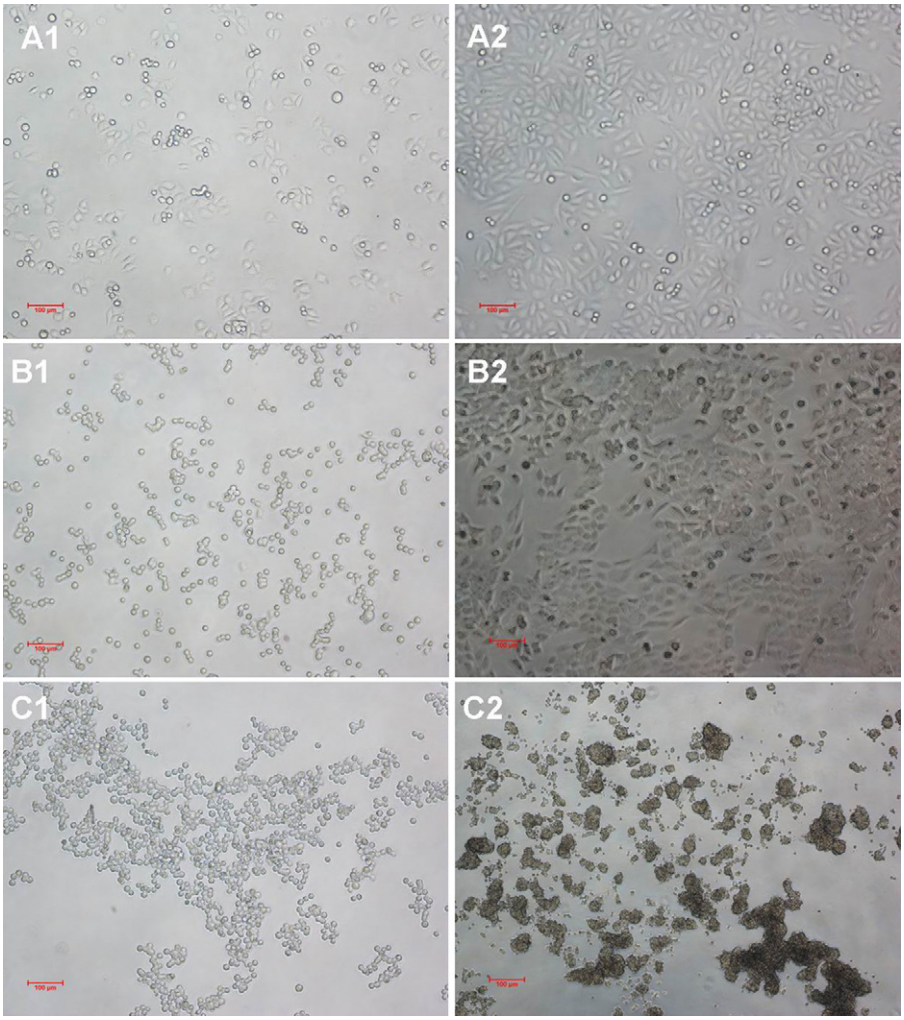

Fig. 3. The morphology of C3A cells on different films. (A1–A2) Cells cultured on TCPs. (B1–B2) Cells cultured on PEMs with a PLL terminal layer. (C1-C2) Cells cultured on PEMs with a ALG terminal layer. (A1, B1, C1) Cells cultured for 0 day. (A2, B2, C2) Cells cultured for 4 day. Bar: 100 μm.

adsorption of proteins on the surface of biomaterials is generally regarded as the trigger event (Wilson et al., 2005). The protein adsorbing will define cellular adhesion and the subsequent biological event. Thus, to better understand cell/biomaterial interaction, the protein adsorption should not be ignored.

Protein adsorption is a complex process, which has been suggested as a result of one or more interactions between proteins and biomaterial surfaces. This process is dependent on the nature of proteins and biomaterials, which is influenced by the microenvironment such as pH and ionic strength (Martins et al., 2010). In this study, the protein adsorption text was conducted at the physiological condition (pH 7.4). Fibrinogen molecule holds an overall negative net charge at a pH of 7.4. Thus, when both substrate and protein adsorption occurs at a pH of 7.4, electrostatic interactions are mainly responsible for fibrinogen deposition since it adsorbs on PLL opposite-charged surface and not on ALG like-charged surface. Protein adsorption is strongly dependent on the charge of the terminating layer.

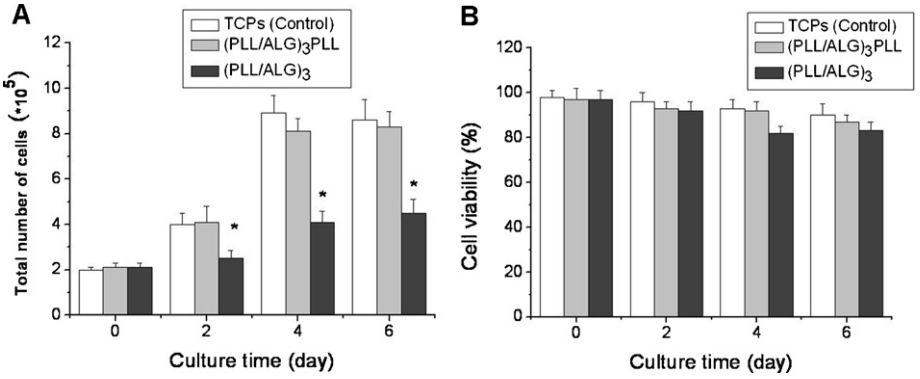

Fig. 4. The growth of cells. (A) The proliferation of cells and (B) the viability of cells (*$p < 0.05$).

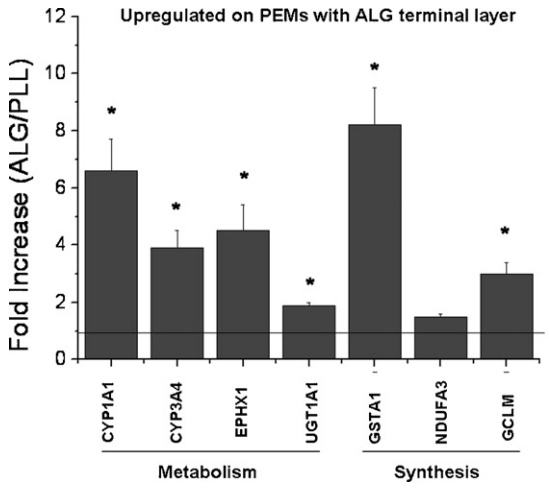

Fig. 5. The functional genes expression of encapsulated C3A cells on PEMs with different terminal layer. Most genes expression of cells on PEMs with a ALG layers is significantly higher that on PEMs with a PLL layers (*$p < 0.05$).

Besides the electrostatic interactions, the protein adsorption may also be influenced by the surface wettability of substrates. It has been reported that the hydrophobic interaction between materials and proteins would not be supported when the contact angle is less than 75° (Vogler, 1999). Therefore, the hydrophobic interaction dose not seem to be the crucial factor for these outcome. As discussed above, even though there may be other potential interactions between the protein and the PEMs, the electrostatic interaction dominated in this process under the scope of this research.

Fibrinogen molecule can combine with the integrin on the cellular membrane. Thus, the adsorption of fibrinogen on the PEMs may further promote the attachment of cells. Our results showed that the attachment of C3A cells was correlated with the adsorption of fibrinogen, which was influenced by the surface charges on the PEMs. In addition, fibrinogen may promote other adsorption of proteins, which can further influence the attachment of cells. With regard to biomaterials used for tissue engineering, the biomaterial interface/surface involves multiprotein system and cells. The protein conformation, the competitive or stimulative behavior of fibrinogen adsorption in the presence of other proteins may participate in this complex process (Martins et al., 2010). However, exploring the response of a single protein is a prerequisite to understand how the properties of the substrate can influence the deposition of proteins and subsequent cell behavior.

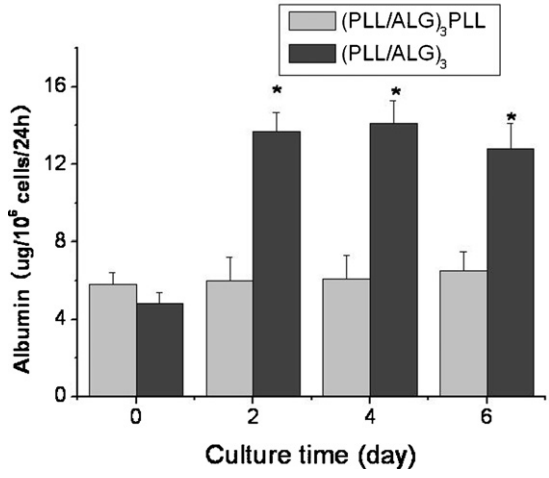

Fig. 6. The albumin secretion of encapsulated C3A cells on PEMs with a ALG layers is significantly higher that on PEMs with a PLL layers (*$p < 0.05$).

In this study, cells on ALG and PLL terminal layer displayed aggregated and spread morphology, respectively. This is mainly determined by the force balance between cell–cell and cell–substrata (Powers, Rodriguez, & Griffith, 1997), which is mediated by the protein adsorption. When cell contractile forces are greater than cell–matrix adhesion forces, spherical aggregates form; when cell contractile forces are weaker than cell–substrata adhesion forces, cells remain essentially spread. In our study, the PLL of the outer layer promotes the proteins adsorption and cell attachment. The ALG of the outer layer may not cover entirely the surface so that the beneath opposite charge chains might still emerge. Thus, the ALG terminal layer exhibited a small number of fibrinogen adsorption and cell attachment. In addition, the expression of most genes involved in xenobiotic metabolism and synthesis is higher on ALG terminal layer of PEMs than that on PLL terminal layer of PEMs. This can be further confirmed by using a protein based detection analysis for albumin secretion. Thus, C3A cells tend to attach, proliferate and form flat monolayer cultures, but exhibit limited function on PEMs with a PLL terminal layer. In contrast, C3A cells aggregate and exhibit improved function, but proliferate lowly on PEMs with a ALG terminal layer. The cell–matrix interaction promotes the growth of cells and the cell–cell interaction maintains the function of cells (Sazonova et al., 2011). One possibility is that the close cell-to-cell contact imposed on proliferating cell colonies may encourage formation of intercellular connections and maintains cell polarity.

5. Conclusion

In summary, we demonstrated that the behavior of C3A cells could be modulated via tuning of surface charges on PEMs, which was different from that via grafting functional groups on PEMs. The zeta potential of $(PLL/ALG)_n$ PEMs showed alternating reversals characteristic of the LbL assembly. Protein adsorption study showed that fibrinogen deposition could be modulated via the terminal layer and the surface charges of PEMs. Furthermore, C3A cells tend to attach, proliferate and form flat monolayer cultures, but exhibit limited function on PLL terminal layer of PEMs. In contrast, C3A cells aggregate and exhibit improved function, but proliferate lowly on ALG terminal layer of PEMs. It suggests that the behavior of C3A cells is correlated with the adsorption of fibrinogen, which is influenced by the surface charges on the PEMs. The results have significant implications for understanding the effect of biomaterials' surface properties on the protein adsorption and behavior of cells, and providing a platform for the future design of tissue engineering.

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References

Berg, M. C., Yang, S. Y., Hammond, P. T., & Rubner, M. F. (2004). Controlling mammalian cell interactions on patterned polyelectrolyte multilayer surfaces. *Langmuir*, *20*, 1362–1368.

Chen, A. A., Khetani, S. R., Lee, S., Bhatia, S. N., & Van Vliet, K. J. (2009). Modulation of hepatocyte phenotype in vitro via chemomechanical tuning of polyelectrolyte multilayers. *Biomaterials*, *30*, 1113–1120.

Chen, Y. M., Ogawa, R., Kakugo, A., Osada, Y., & Gong, J. P. (2009). Dynamic cell behavior on synthetic hydrogels with different charge densities. *Soft Matter*, *5*, 1804–1811.

Elkayam, T., Amitay-shaprut, S., Dvir-ginzberg, M., Harel, T., & Cohen, S. (2006). Enhancing the drug metabolism activities of C3A – A human hepatocyte cell line – By tissue engineering within alginate scaffolds. *Tissue Engineering*, *12*, 1357–1368.

Glicklis, R., Shapiro, L., Agbaria, R., Merchuk, J. C., & Cohen, S. (2000). Hepatocyte behavior within three-dimensional porous alginate scaffolds. *Biotechnology and Bioengineering*, *67*, 344–353.

Griffith, L. G., & Swartz, M. A. (2006). Capturing complex 3D tissue physiology in vitro. *Nature Reviews Molecular Cell Biology*, *7*, 211–224.

Jessel, N., Atalar, F., Lavalle, P., Mutterer, J., Decher, G., Schaaf, P., et al. (2003). Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. *Advanced Materials*, *15*, 692–695.

Khalil, M., Shariat-Panahi, A., Tootle, R., Ryder, T., McCloskey, P., Roberts, E., et al. (2001). Human hepatocyte cell lines proliferating as cohesive spheroid colonies in alginate markedly upregulate both synthetic and detoxificatory liver function. *Journal of Hepatology*, *34*, 68–77.

Kim, B. S., Park, I. K., Hoshiba, T., Jiang, H. L., Choi, Y. J., Akaike, T., et al. (2011). Design of artificial extracellular matrices for tissue engineering. *Progress in Polymer Science*, *36*, 238–268.

Lozoya, O. A., Wauthier, E., Turner, R. A., Barbier, C., Prestwich, G. D., Guilak, F., et al. (2011). Regulation of hepatic stem/progenitor phenotype by microenvironment stiffness in hydrogel models of the human liver stem cell niche. *Biomaterials*, *32*, 7042–7389.

Martins, G. V., Merino, E. G., Mano, J. F., & Alves, N. M. (2010). Crosslink effect and albumin adsorption onto chitosan/alginate multilayered systems: An in situ QCM-D study. *Macromolecular Bioscience*, *10*, 1444–1455.

Nagavasu, A., Yokoi, H., Minaquchi, J. A., Hosaka, Y. Z., Ueda, H., & Takehana, K. (2012). Efficacy of self-assembled hydrogels composed of positively or negatively charged peptides as scaffolds for cell culture. *Journal of Biomaterials Applications*, *26*, 651–665.

Ng, S., Wu, Y. N., Zhou, Y., Toh, Y. E., Ho, Z. Z., Chia, S. M., et al. (2005). Optimization of 3-D hepatocyte culture by controlling the physical and chemical properties of the extra-cellular matrices. *Biomaterials*, *26*, 3153–3163.

Picart, C., Elkaim, R., Richert, L., Audoin, F., Arntz, Y., Da Silva Cardoso, M., et al. (2005). Primary cell adhesion on RGD-functionalized and covalently crosslinked thin polyelectrolyte multilayer films. *Advanced Functional Materials*, *15*, 648–654.

Powers, M. J., Rodriguez, R. E., & Griffith, L. G. (1997). Cell–substratum adhesion strength as a determinant of hepatocyte aggregate morphology. *Biotechnology and Bioengineering*, *53*, 415–426.

Richert, L., Boulmedais, F., Lavalle, P., Mutterer, J., Ferreux, E., Decher, G., et al. (2004). Improvement of stability and cell adhesion properties of polyelectrolyte multilayer films by chemical cross-linking. *Biomacromolecules*, *5*, 284–294.

Richert, L., Lavalle, P., Vautier, D., Senger, B., Stoltz, J. F., Schaaf, P., et al. (2002). Cell interactions with polyelectrolyte multilayer films. *Biomacromolecules*, *3*, 1170–1178.

Sazonova, O. V., Lee, K. L., Isenberg, B. C., Rich, C. B., Nugent, M. A., & Wong, J. Y. (2011). Cell–cell interactions mediate the response of vascular smooth muscle cells to substrate stiffness. *Biophysical Journal*, *3*, 622–630.

Schneider, G. B., English, A., Abraham, M., Zaharias, R., Stanford, C., & Keller, J. (2004). The effect of hydrogel charge density on cell attachment. *Biomaterials*, *25*, 3023–3028.

Schrader, J., Gordon-Walker, T. T., Aucott, R. L., Deemter, M., Quaas, A., Walsh, S., et al. (2011). Matrix stiffness modulates proliferation, chemotherapeutic response and dormancy in hepatocellular carcinoma cells. *Hepatology*, *53*, 1192–1205.

Seo, S. J., Akaike, T., Choi, Y. J., Shirakawa, M., Kang, I. K., & Cho, C. S. (2005). Alginate microcapsules prepared with xyloglucan as a synthetic extracellur matrix for hepatocyte attachment. *Biomaterials*, *26*, 3607–3615.

Stabenfeldt, S. E., & Laplaca, M. C. (2011). Variations in rigidity and ligand density influence neuronal response in methylcellulose-laminin hydrogels. *Acta Biomaterialia*, *7*, 4102–4108.

Strain, A. J., & Neuberger, J. M. (2002). A bioartificial liver – State of the art. *Science*, *295*, 1005–1009.

Vogler, E. A. (1999). Water and the acute biological response to surfaces. *Journal of Biomaterials Science, Polymer Edition*, *10*, 1015–1045.

Wilson, C. J., Clegg, R. E., Leavesley, D. I., & Pearcy, M. J. (2005). Mediation of biomaterial–cell interactions by adsorbed proteins: A review. *Tissue Engineering*, *11*, 1–18.

Wittmer, C. R., Phelps, J. A., Lepus, C. M., Saltzman, W. M., Harding, M. J., & Van Tassel, P. R. (2008). Multilayer nanofilms as substrates for hepatocellular applications. *Biomaterials*, *29*, 4082–4090.

Xie, H. G., Li, X. X., Lv, G. J., Xie, W. Y., Zhu, J., Luxbacher, T., et al. (2010). Effect of surface wettability and charge on protein adsorption onto implantable alginate-chitosan-alginate microcapsule surfaces. *Journal of Biomedical Materials Research A*, *92A*, 1357–1365.

Xie, H. G., Zheng, J. N., Li, X. X., Liu, X. D., Zhu, J., Wang, F., et al. (2010). Effect of surface morphology and charge on the amount and conformation of fibrinogen adsorbed onto alginate/chitosan microcapsules. *Langmuir*, *26*, 5587–5594.

Yamada, K. M., & Cukierman, E. (2007). Modeling tissue morphogenesis and cancer in 3D. *Cell*, *130*, 601–610.