Colloids and Surfaces B: Biointerfaces 116 (2014) 459–464

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Cell adhesion on supported lipid bilayers functionalized with RGD peptides monitored by using a quartz crystal microbalance with dissipation

Xiaohua Zhu[a,b], Zhao Wang[a,b], Ansha Zhao[a,b,*], Nan Huang[a,b], Huadong Chen[c], Shuo Zhou[a,b], Xiao Xie[d]

[a] Key Laboratory of Advanced Technologies of Materials, Ministry of Education, Southwest Jiaotong University, Chengdu 610031, China
[b] School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China
[c] School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China
[d] State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China

ARTICLE INFO

Article history:
Received 13 October 2013
Received in revised form 2 January 2014
Accepted 22 January 2014
Available online 30 January 2014

Keywords:
SLBs
QCM-D
Cell adhesion
RGD
Dynamic monitoring

ABSTRACT

Supported lipid bilayer (SLB) is one of the most widely used structures to mimic cell membranes. To study the cell–cell, cell–matrix and cell–material interactions, supported lipid bilayers (SLBs) functionalized with RGD peptides (SLBs–RGD) were prepared by vesicle fusion on a SiO_2 quartz crystal, and subsequently bone mesenchymal stem cells (BMSCs) adhesion was analyzed. A quartz crystal microbalance with dissipation (QCM-D) was utilized to detect the dynamic adsorption behavior of lipid vesicles and BMSCs in real time. Observations obtained by QCM-D signals are confirmed by conducting fluorescence microscopy.

QCM-D measurements showed the SLB formation starts at the critical concentration of the vesicles. More BMSCs adhered on SLBs–RGD than on SLBs. With the presence of SLBs, the adhesion cells on SLBs surfaces had a rounded morphology, and cells on SLBs–RGD will take long time to rearrange their cytoskeleton, which led to incomplete spreading compared with SiO_2. Differences in adhesion density and adhesion properties of the cells on the different substrates could be traced at the dissipation versus frequency ($\Delta D/\Delta f$) plots. These results indicate that RGD in/on SLBs could provide anchorage sites for more cells adhesion. QCM-D is demonstrated to be a useful tool for evaluating the interactions between various biological and non-biological systems in situ and in real-time.

1. Introduction

Cell membrane is not only the dynamic barrier between intracellular environment and extracellular matrix, but also the place of signal transduction and material exchange. To study the interaction of cell–cell, cell–matrix and cell–material, we should be aware of the structure of the cell membrane. For a long time, the fluid mosaic model [1] was regarded to present the structure of cell membranes. However, the cell membrane structure is still too complex for researchers to study its function. Therefore, a simplified cell membrane structure model has been obtained more attention. The emergence of supported lipid bilayers (SLBs) provided a simplified platform for molecular biology researching [2–7]. With structure and dynamic properties like cell membrane, simplicity of fabrication, composing of single components or defined lipid mixture [6,8–12], SLBs are largely used as biomimetic model systems in the study of biophysical and biochemical properties of biological membranes [5,13]. Similar to the cell membrane, SLBs are composed of some defined proteins to form cell adhesion sites, just like rafts structures [14]. They can be used to study cellular interactions. Pierschbacher et al. determined an essential sequence (Arg-Gly-Asp-Ser, RGD) which carries cell attachment-promoting activity in fibronectin [15]. The sequence had also been found in other extracellular matrices proteins and blood proteins, such as vitronectin, osteopontin, collagens, thrombospondin, fibrinogen, and von Willebrand factor. Meanwhile, the RGD sequence was thought as cell recognition site of these proteins [16]. In the bionics field, the SLBs were also functionalized with RGD peptides to achieve the goal of mimicking cell membranes. Ananthanarayanan et al. used phospholipids bilayers surface functionalized with RGD peptides to study the adhesion and proliferation of neural stem cell (NSC). They demonstrated that phospholipids bilayers surface functionalized with RGD peptides supported NSC adhesion, proliferation, and differentiation into mature neural cells [17].

* Corresponding author at: Key Laboratory of Advanced Technologies of Materials, Ministry of Education, Southwest Jiaotong University., No. 111, North One Erhuan Road, Chengdu, China. Tel.: +86 28 8763 4148.
E-mail address: anshazhao@263.net (A. Zhao).

0927-7765/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.colsurfb.2014.01.032

Quartz crystal microbalance with dissipation (QCM-D) is a device which based on the principle of piezoelectric effect to characterize the change of frequency and dissipation in the liquid phase. An increase of mass on the surface of the quartz crystal sensor leads to a decrease of frequency. Hence, it is a very sensitive mass testing instrument access to the nanogram level. Moreover, the dissipation change measured by QCM-D indicates the change of viscoelasticity and can be applied to describe the biomolecular structure change at the interface, for example, protein and vesicle adsorption [11,18–21], lipid bilayers formation [22–27], cell adhesion and spreading [28–36]. So these characterizations enable QCM-D to be an attractive technique in the molecular biology [37,38], biochemical/chemical [39–42], and biomedical area [43,44].

Previously [30–32,35,36], researchers have studied cell adhesion by using QCM-D. But these adhesion surfaces, which cells attached, were either metal surfaces or polymer surfaces. And these surfaces cannot mimic the cell membrane as well as lipid bilayer. Although some researchers have reported cell adhesion on SLBs [17,45–47], these studies were performed in static condition and cannot describe the whole adhesion process in real time. In this article, SLB functionalized with RGD peptides was formed by vesicle fusion [48] to mimic cell membranes. SLBs–RGD formation and interaction with bone mesenchymal stem cells (BMSCs) were monitored by QCM-D in real time in order to understand the mechanism of assembled lipid bilayers and the interaction with adhesion cells.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] dodecanoyl]-*sn*-glycero-3-phosphocholine was used as the lipid purchased from Avanti Polar Lipids Inc., USA. The RGD peptides (Fmoc-GGGNGEPRGDTYRAY-NH2 [17], 95%) used in this article were purchased from GL Biochem Ltd., Shanghai, China. LiposoFast-Basic was used to extrude lipids to vesicles purchased from AVESTIN, Inc., Canada. BMSCs were isolated from the femurs of a SD rat (female, 100 g, within two weeks old) by using density gradient centrifugation method. DMEM/F-12 (1:1) culture media for BMSCs was purchased from Hyclone, USA. QCM-D instrument (E4, flow moduel) was utilized to monitor cell adhesion on SLB in real-time purchased from Q-sense AB, Sweden.

2.2. Methods

2.2.1. Preparation of liposomes

Lipids were mixed with chloroform to a concentration of 0.25 mg/ml. 200 μl of mixed solution was added into a 50 ml flask and evaporated with a rotary vacuum pump. Then, 42 μl RGD peptides solution (3.5 mg/ml) was added into the flask and evaporated in a rotary evaporator (Shenshun Bio-Technology Ltd., Shanghai, China) until lipids, with RGD peptides, formed layers of uniform film. Subsequently, 1 ml TBS buffer solution (tris:NaCl:deionized water = 2.422 g:17.532 g:1000 ml, pH 7.4) was added into the same flask. Ultrasound treating the flask until the lipid film dissolved. The same method was used to prepare another kind of lipid sample which without RGD peptides. These two kinds of lipid solution were extruded by an extruder through a 100 nm filter membrane for 31 times. 20 μl of these two kinds of samples were mixed with 1 ml TBS buffer, respectively, for next experiment.

2.2.2. Cell adhesion on SLBs

Vesicle fusion method was utilized to prepare SLBs (Fig. 1). The two kinds of liposome solution mentioned at the end of 2.2.1 were dropped on glass, then, incubated for 30 min at 37 °C. After that, these two kinds of samples were washed by TBS buffer gently.

The BMSCs (10^4 cells/cm^2) adhesion test was performed on glass, SLBs and SLBs–RGD for 2 h. Four parallel samples were prepared. The adherent BMSCs were fixed with 2.5% glutaraldehyde after 2 h, then, stained with rhodamine and DAPI. Fluoresce observation and cell counting had been done at last. Statistical analysis was done by using the method of Li et al. [49]. $P < 0.05$ was considered as a significant difference.

2.2.3. QCM-D detection

SiO_2 quartz crystal sensors were cleaned with sodium dodecyl sulfate (SDS) solution (2% w/w), ethanol and deionized water, respectively. Each washing step was treated for 10 min by an ultrasonic cleaner. After that, the crystal sensors were dried with a gentle stream of nitrogen and radiated by ultraviolet for 30 min.

The crystal sensors were inserted into the QCM-D instrument, and then TBS buffer was introduced into the QCM-D's three channels with a speed of 30 μl/min to run base lines. After the base lines were stable, the two kinds of lipid vesicle solution and TBS buffer were introduced into the different channels, respectively. After the SLBs/SLBs–RGD had been formed, TBS buffer was injected to the QCM-D chamber to rinse the sensor. Then, Serum-free DMEM/F-12 medium was introduced into the channels. When the signals were stable, BMSCs with serum-free DMEM/F-12 medium were introduced into the channels to monitor cells adhesion. One hour later, the pump was stopped and kept off for 2 h. After that, non-serum medium solution was injected into the channels until the signal was stable.

3. Results and discussion

3.1. Formation of SLBs

Vesicle fusion is one of the most common methods for SLBs preparation. Fig. 1 shows the schematic of SLB formation via

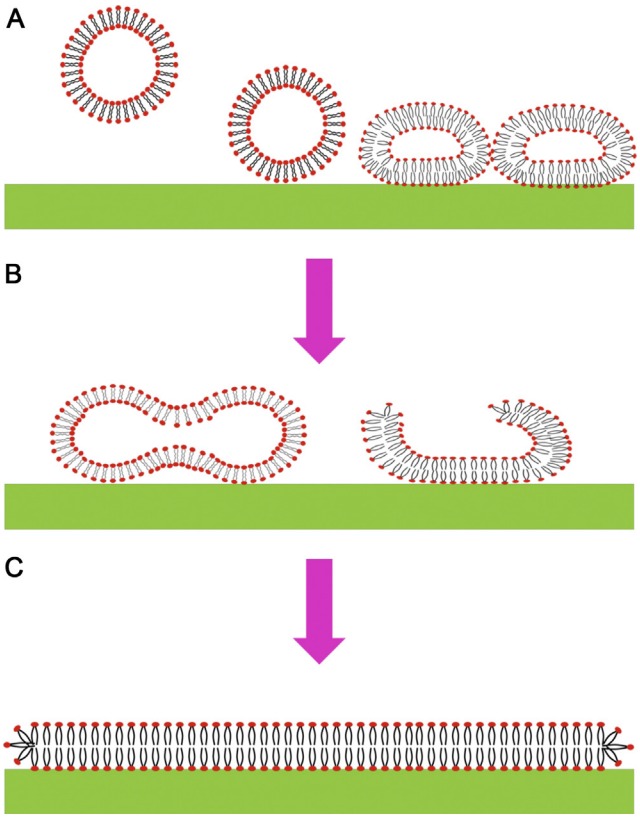

Fig. 1. Schematic illustration of SLBs preparation via vesicle fusion.