Biochemical Engineering Journal 117 (2017) 82–88

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

# Investigation of cellular response to covalent immobilization of peptide and hydrophobic attachment of peptide amphiphiles on substrates

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ARTICLE INFO

*Article history:*
Received 17 May 2016
Received in revised form
25 September 2016
Accepted 16 October 2016
Available online 5 November 2016

*Keywords:*
RGD peptide
Lipidated peptide
Silicon surface
Covalent immobilization
Cell response
Alkyl tail

ABSTRACT

An overall investigation was made of the cell morphology, adhesion, viability, and proliferation of human fibroblast cells on surfaces functionalized with peptide and peptide amphiphiles. We compared surfaces in which the RGD peptide was immobilized covalently onto silicon with those in which lipidated versions of the RGD peptide were hydrophobically attached to the alkylated silicon surfaces. The hydrophobically attached peptide amphiphile on alkylated silicon surfaces produce structures that are somewhat akin to the structure of a cell membrane. Scanning electron microscopy (SEM) and Laser scanning confocal microscopy (LSCM) were used to characterize the seeding human fibroblast cells on all prepared surfaces. Surfaces were also evaluated with a methyl tetrazole sulfate (MTS) assay to compare the proliferation ability. Cell-substrate interactions were examined through cell adhesion assay. Peptide-amphiphile modified surfaces exhibited substantially superior cellular responses compared to those on the covalently immobilized peptide. It was also shown that the length of alkyl tail in lipidated peptides may influence cellular response. Hydrophobically attached peptide amphiphiles on alkylated silicon surfaces may suggest new biomimetic platforms for further studies of the interaction between cells and extracellular matrix.
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## 1. Introduction

There is a significant interest in the development of biomimetic materials that can mimic extracellular matrix (ECM). Cell to cell and cell to ECM interactions are important for the formation of in vivo tissues and organs [1–7]. Therefore, the production of a stable, functional, and bioactive film is of interest for applications ranging from biosensors, biochip development, and tissue engineering [8–10]. In early research, long chain of ECM components such as fibronectin and vitronectin was used for surface modifications of inorganic substrates for this purpose [11,12]. However, it is now known that interaction between ECM and cell membrane receptors occurs via only short segments of ECM [13,14]. One of these sequences is the RGD sequence and is now known to promote cell adhesion on inorganic or polymeric surfaces [11,15–20]. Cell adhesion is mediated by integrins through a cascade of four different events including cell attachment, spreading, organization of actin cytoskeleton, and formation of focal adhesions [16,21]. During the adhesion step of cells, integrins undergo several conformational alterations throughout ligand binding and transmembrane signaling [22,23].

Different methods including bioconjugation, chemoselective ligation, and click chemistry have been used to covalently immobilize an RGD peptide on different substrates [24–33]. The techniques used for peptide immobilization must ensure that the attachment site does not interfere with the accessibility of the active site and that active sites remain active during attachment with a high surface density of the peptide. Molecular self-assembly is a powerful approach to design such a model that satisfies some of these requirements. The best known of such self-assembled structures is a lipidated peptide which is achieved by attaching a hydrophobic moiety to a peptide to create an amphiphilic molecule [8,34,35]. The hydrophobic tail serves to orient the molecules while the peptide head group imparts the specific biological activity [6,36]. Previous studies have shown that the lipidated peptide amphiphiles (PAs) have potential applications in tissue engineering and drug delivery [37–39]. Stroumpoulis et al. [40] reported an effective method to screen biological probes for cell adhesion and growth through the incorporation of PAs gradients in a membrane environment. Unique biomimetic materials have been

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http://dx.doi.org/10.1016/j.bej.2016.10.023
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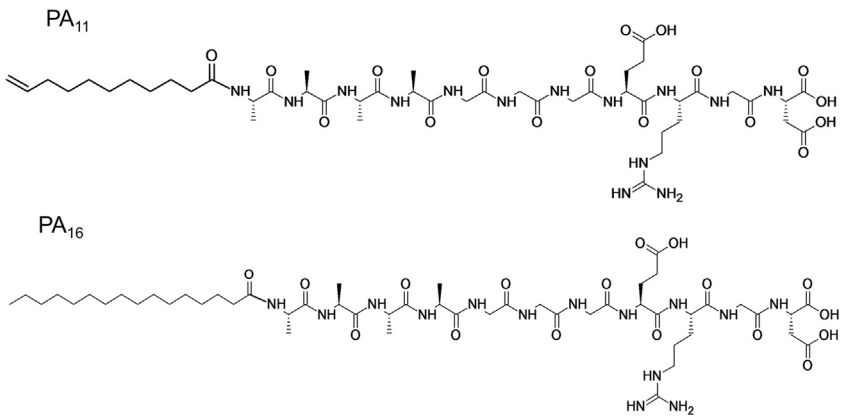


**Fig. 1.** The chemical structure of undecanoyl and palmitoyl peptide.

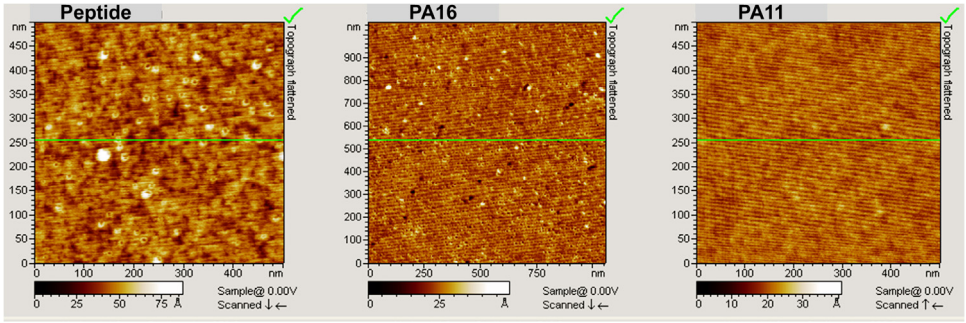


**Fig. 2.** AFM images of peptide, $PA_{16}$ and $PA_{11}$ on alkyllated silicon surfaces.

developed through the incorporation of lipidated peptides into self-assembled structures such as films [8]. In a previous research [41] we synthesized and characterized two derivatives of PAs including undecanoyl lipidated peptide (PA11) and palmitoyl lipidated peptide (PA16) (Fig. 1). TEM observations revealed [41] the presence of a network of nanofibers for PA16 and PA11. We developed bilayer surfaces with restricted fluidity via covalent attachment of tetradecane monolayers on hydrolyzed surfaces in a self-assembly method. Hydrophobic attachment of PA on these surfaces then produced bilayer structures composed of a hydrophobic interior and a hydrophilic exterior.

In this paper, we compare cellular responses of the hydrophobically attached PA on the alkylated surfaces with the covalently immobilized peptide on silicon surfaces. It should be noted that the same sequence of amino acids (A-A-A-A-G-G-G-E-R-G-D) were used in both the peptide and the PAs.

## 2. Experimental methods

### 2.1. Materials

Human dermal fibroblasts (HDF; line GM3348) were obtained from Sigma-Aldrich. All chemicals were purchased from Sigma-Aldrich (Sydney, NSW, Australia) and used as received without further purification. Milli- Q water (18 MΩ cm) was used for the rinsing and preparation of solutions.

### 2.2. Synthesis of peptide and peptide amphiphiles

Peptide synthesis was performed on a PS3 automated peptide synthesizer which is described in the previous paper [25]. To produce the lipidated form of the peptide, it was acylated with a mixture of either 0.5 mmol palmitic acid or undecanoic acid, 0.5 mmol *N*-[(1H-benzotriazol-1-yl)(dimethylamino) methylene]-*N*-methyl-methan aminimum hexafluorophosphates N-oxide, 1 mmol diisopropyl methylamine and 5 ml Dimethylformamide which has been described in detail in a previous paper [41]. Mass spectra for peptide, PA11and PA16 were 931.53*m*/*z* $[M+H]^+$,1086 *m*/*z* $[M+H]^+$ and 1012 *m*/*z* $[M+H]^+$, respectively. The crude peptide and peptide amphiphiles were analyzed by HPLC and it was found that all of them had a purity of >95%.

### 2.3. Immobilization of RGD peptide on the surface of silicon

The chemical attachment of the peptides onto the silicon surface was performed as follows. An N hydroxyl succinimide (NHS) ester terminated organic monolayer film was first produced using a self-assembly method [42]. 100 mg of the peptide was attached to this NHS terminated self-assembled monolayer by incubating it in 10 ml phosphate buffered saline (PBS) at pH 7 for 3 h with 2 min sonication every 15 min. The samples were then rinsed consecutively with copious volumes of chloroform, ethyl acetate, ethanol, and water. Details of this procedure are given in reference [25].

### 2.4. Hydrophobic attachment of peptide amphiphiles on alkylated silicon surfaces

The process begins by removal of the oxide layer from the silicon surface using 40% ammonium fluoride which yields a hydroxylated surface. The wafer is then immersed in tetradecane and exposed to the ultraviolet light. The terminal double bond then links to the hydroxylated surface to yield tetradecane covalently bonded to the silicon. Detail of this process has been given elsewhere [42]. Formation of the hydrophobically attached films of the lipidated peptide amphiphiles onto alkylated silicon surface involved incubation of the alkane functionalized silicon surface in 100 mg lipidated pep-

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