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Cell adhesion on an artificial extracellular matrix using aptamer-functionalized PEG hydrogels

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ABSTRACT

The development of an artificial extracellular matrix (ECM) is important to regenerative medicine because the ECM plays complex and dynamic roles in the regulation of cell behavior. In this study, nucleic acid aptamers were applied to functionalize hydrogels for mimicking the adhesion sites of the ECM. The results showed that nucleic acid aptamers could be incorporated into polyethylene glycol (PEG) hydrogels via free radical polymerization. The incorporation of the aptamers produced only a moderate effect on the mechanical properties of the PEG hydrogels. Importantly, the results also showed that the aptamers effectively induced cell type-specific adhesion to the PEG hydrogels without affecting cell viability. The cell adhesion was a function of the aptamer concentration, the spacer length and the cell seeding time. In addition, cell adhesion to the aptamer-functionalized hydrogel could be attenuated by means of aptamer inactivation in a physiological condition. Thus, aptamer-functionalized hydrogels are promising biomaterials for the development of artificial ECMs.

1. Introduction

The integration of bioactive molecules and/or living cells into synthetic materials has led to the development of a variety of biomimetic and bioinspired materials [1–3]. The study of artificial ECMs has particularly attracted significant attention because of their potential applications in the field of regenerative medicine [3–5]. Natural ECMs are comprised of insoluble macromolecules (*e.g.,* proteoglycans), soluble signaling molecules (*e.g.,* growth factors), and adhesion ligands (*e.g.,* fibronectin) [6]. These ECM components play complex and dynamic roles in tissues. Based on the communication with these components, cells acquire essential biochemical and biophysical cues that determine the behavior of the cells [6]. Hydrogels have been widely studied as a fundamental support component for the synthesis of artificial ECMs [7,8], because they have structural similarities to human tissues with tunable viscoelasticity and high permeability for molecular transport. However, most hydrogels do not possess the essential biochemical and/or biophysical cues required for regulating cell behavior. It is therefore necessary to incorporate biofunctional molecules into the hydrogel network to simulate the natural regulatory processes of ECMs.

The dissolution of signaling molecules (*e.g.,* growth factors) into a hydrogel is a common way to incorporate biochemical cues into an artificial extracellular matrix [9]. However, most hydrogels are highly permeable in nature. Though this characteristic is advantageous for the molecular transport of nutrients and wastes to support cell survival and intercellular communication, it is disadvantageous for the long-term release of signaling molecules to provide cells with sufficient biochemical stimuli. To solve this problem, polymeric microparticles that can control the release of growth factors have been used to functionalize hydrogels [10]. Alternatively, hydrogels can be modified with heparin, Ni^{2+}, or peptides that can physically absorb positively charged or histidine-tagged signaling molecules to achieve the sustained release [11–13]. Hydrogels can also be functionalized with nucleic acid aptamers to control the release of growth factors [14–17]. Nucleic acid aptamers are single-stranded oligonucleotides selected from the libraries of synthetic oligonucleotides [18,19]. They have numerous merits such as high affinity, high specificity, and little immunogenicity. In addition, they can be chemically modified to acquire resistance against nuclease degradation [20]. Studies also show that the release of growth factors from aptamer-functionalized hydrogels can be triggered by PEGylated complementary oligonucleotides at predetermined multiple time points [16]. Therefore, it is promising that multiple

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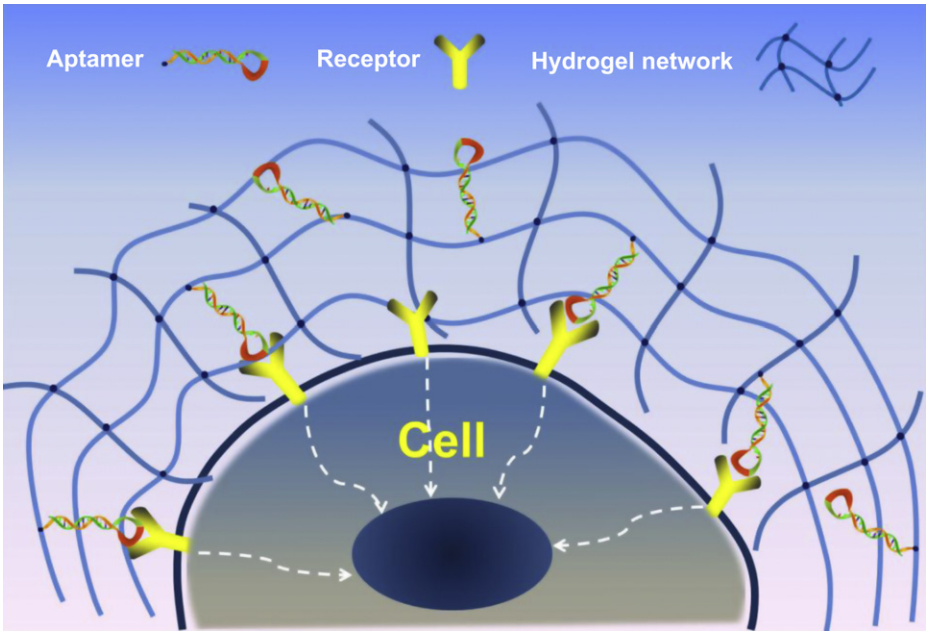

Fig. 1. Schematic of cell adhesion to aptamer-functionalized hydrogel.

growth factors can be incorporated into aptamer-functionalized hydrogels as biochemical cues to mimic the sustained and/or triggered release functionality of ECMs.

In addition to the necessity of incorporating soluble signaling molecules into the hydrogels, it is also important to functionalize hydrogels with adhesion ligands to provide cells with biophysical cues. Natural adhesion proteins or small adhesion peptide sequences identified within the adhesion proteins have been used to modify hydrogels for this purpose [3]. Though natural adhesion proteins can provide cells with an environment similar to the native extracellular microenvironment, they are often limited by fast degradation [21]. In addition, the proteins may lose the capability of recognizing their cognate receptors during the material synthesis. For instance, collagen is one of the critical components in the ECM, interacting with numerous cell surface receptors [22]. However, recent experimental results have indicated that collagen sponges have to be further functionalized with adhesion peptides to improve their cell recognition function [23]. Small adhesion peptides have merits such as easy synthesis, easy conjugation, and little immunogenicity, but they often require circularization, multimerization, and presentation in the context of "parent" proteins in order to achieve sufficient structural stability and binding affinity [21]. Therefore, it is desirable to explore new biomolecular ligands to functionalize hydrogels and mimic the biophysical cues of ECMs.

The long-term goal of this study is to develop an artificial ECM using hydrogels and nucleic acid aptamers. The aptamers are expected to not only control the release of multiple growth factors (*i.e.*, biochemical cues), but also provide cells with cell binding sites (*i.e.*, biophysical cues). Because our previous research demonstrated that aptamer-functionalized hydrogels could allow for the temporal control of growth factors with desired release kinetics [14–17], this study was focused on studying the feasibility of using aptamers as the binding sites of hydrogels for cell adhesion (Fig. 1). PEG hydrogels were used as the model material system due to their biocompatibility and their wide applications in the field of biomaterials [24]. Aptamers were incorporated into the PEG hydrogels through free radical polymerization. The PEG hydrogels were characterized by rheology, fluorescence microscopy, and a swelling test. The effects of various parameters on cell adhesion to the aptamer-functionalized PEG hydrogels were also studied.

2. Materials and methods

2.1. Chemical reagents

Poly(ethylene glycol) diacrylate (PEGDA; average M_n: 700 Da) and 3-(trimethoxysilyl)propyl methacrylate (TMSPM) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate buffered saline (PBS), ammonium persulfate (APS), N,N,N′,N′-tetramethylenediamine (TEMED), and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Suwanee, GA). All oligonucleotides (Table 1) were purchased from Integrated DNA Technologies (Coralville, IA).

2.2. Prediction of secondary structures

The secondary structures of the aptamers were generated by using RNAstructure version 5.0 (http://rna.urmc.rochester.edu/rnastructure.html). The predicted structures with the lowest free energies were presented.

2.3. Synthesis of PEG hydrogels

PEG hydrogels were synthesized on a glass surface for the convenience of operating cell adhesion experiments. Glass slides were cut to the dimension of approximately 8 mm × 8 mm. The glass was cleaned using acetone and 1 M NaOH solution sequentially. Acrylate groups were generated on the glass surface through silanization as previously described [25]. In brief, the silanization solution was prepared by mixing 0.5 mL TMSPM in 50 mL ethanol with 1.5 mL of 10% diluted glacial acetic acid. After the

Table 1
Sequences of oligonucleotides.

Name	Sequence (5′→3′)
sgc8c-10A	/*acrydite*/AAAAAAAAAATCTAACTGCTGCGCCGCCG GGAAAATACTGTACGGTTAGA
sgc8c-5A	/*acrydite*/-AAAAATCTAACTGCTGCGCCGCCGGGAA AATACTGTACGGTTAGA
sgc8c-0A	/*acrydite*/-TCTAACTGCTGCGCCGCCGGGAAAATAC TGTACGGTTAGA
ACA	/*acrydite*/-GCGATACTCCACAGGCTACGGCACGTAG AGCATCACCATGATCCTG
FAW	CACTTAGAGTTCTAACTGCTGCGCCGCCGGGAAAAT ACTGTACGGTTAGA
CO-FAM	/*FAM*/-TCTAACCGTACAGTATTTTC
sgc8c-FAM	/*FAM*/-TCTAACTGCTGCGCCGCCGGGAAAATACTGTA CGGTTAGA
CO	TCTAACCGTACAGTATTTTC

The binding region of the aptamers is underlined. ACA: acrydited control aptamer; FAW: functional aptamer without acrydite.