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Development of peptide-functionalized synthetic hydrogel microarrays for stem cell and tissue engineering applications

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ABSTRACT

Synthetic polymer microarray technology holds remarkable promise to rapidly identify suitable biomaterials for stem cell and tissue engineering applications. However, most of previous microarrayed synthetic polymers do not possess biological ligands (e.g., peptides) to directly engage cell surface receptors. Here, we report the development of peptide-functionalized hydrogel microarrays based on light-assisted copolymerization of poly(ethylene glycol) diacrylates (PEGDA) and methacrylated-peptides. Using solid-phase peptide/organic synthesis, we developed an efficient route to synthesize methacrylated-peptides. In parallel, we identified PEG hydrogels that effectively inhibit non-specific cell adhesion by using PEGDA-700 (M. W. = 700) as a monomer. The combined use of these chemistries enables the development of a powerful platform to prepare peptide-functionalized PEG hydrogel microarrays. Additionally, we identified a linker composed of 4 glycines to ensure sufficient exposure of the peptide moieties from hydrogel surfaces. Further, we used this system to directly compare cell adhesion abilities of several related RGD peptides: *RGD*, *RGDS*, *RGDSG* and *RGDSP*. Finally, we combined the peptide-functionalized hydrogel technology with bioinformatics to construct a library composed of 12 different RGD peptides, including 6 unexplored RGD peptides, to develop culture substrates for hiPSC-derived cardiomyocytes (hiPSC-CMs), a cell type known for poor adhesion to synthetic substrates. 2 out of 6 unexplored RGD peptides showed substantial activities to support hiPSC-CMs. Among them, PMQKM*RGD*VFSP from laminin β4 subunit was found to support the highest adhesion and sarcomere formation of hiPSC-CMs. With bioinformatics, the peptide-functionalized hydrogel microarrays accelerate the discovery of novel biological ligands to develop biomaterials for stem cell and tissue engineering applications.

Statement of Significance

In this manuscript, we described the development of a robust approach to prepare peptide-functionalized synthetic hydrogel microarrays. Combined with bioinformatics, this technology enables us to rapidly identify novel biological ligands for the development of the next generation of functional biomaterials for stem cell and tissue engineering applications.

1. Introduction

Polymer array technology has emerged as a powerful tool for the rapid identification of suitable materials for a variety of stem cell and tissue engineering applications [1–7]. To fabricate synthetic polymer microarrays, photopolymerization of (meth)-acrylates has been extensively utilized [4,8–14]. This is due to their high polymerization rate, as well as the high solubility of (meth)-acrylates in high boiling point organic solvents (e.g., DMF). While this strategy allows for the preparation of synthetic polymers with diversified properties, these polymers usually do not contain biological ligands to directly interact with cell surface receptors (e.g., integrin and

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growth factor receptors) [7,15]. The biological functions of these polymers usually depend upon the serum/extracellular matrix (ECM) proteins adsorbed on their surface from pre-conditioning solution and/or cell culture media [5,10].

To develop substrates capable of interacting with (stem) cell surface receptors in a defined manner, a popular approach is to use functional fragments of ECM proteins (e.g., RGD-peptides) to functionalize the substrates [16–18]. In particular, self-assembled monolayer (SAM) technology has received significant attention as it allows for versatile peptide functionalization strategies [19]. To this end, Kiessling and coworkers prepared thiolated peptides and spotted them onto gold-coated glass slides to prepare peptide-functionalized SAM microarrays [17,20,21]. Alternatively, Kilian and coworkers have taken advantage of recent advances in "click" chemistry [22]. They synthesized the alkyne group functionalized peptides and robotically spotted them with azide-terminated alkanethiolate to prepare peptide microarrays. Another approach to prepare peptide-functionalized SAM microarrays has been developed by Murphy and coworkers [18]. They leveraged the carbodiimide catalyzed conjugation reactions between the n-terminal primary amine of peptides and carboxylic acid terminated SAMs to prepare peptide microarrays [19,23].

In addition to the SAM microarrays, the fabrication of peptide-functionalized hydrogel microarrays has been explored. Unlike with SAMs, hydrogels allow for the easy modulation of the physical properties (e.g., elasticity) of substrates, which has been shown to have controlling effects on (stem) cells [24–26]. For example, Engler and coworkers have shown to influence the differentiation of human mesenchymal stem cells (hMSCs) through the fabrication of hydrogels that closely replicate *in vivo* tissue elasticity [27]. Their results demonstrated that the gels with stiffness similar to muscle elasticity led to myogenic differentiation, while the gels similar to calcified bone led to osteogenic differentiation. To combine the advantages of hydrogels and high-throughput microarray technology, the peptide functionalized hydrogel microarray has been explored. To this end, Hawker and coworkers have developed a versatile synthetic route to prepare peptide-functionalized hydrogel microarrays using thiol-ene chemistry [28]. Despite this progress, the chemistry employed to prepare peptide-functionalized hydrogel microarrays usually involves complicated/inefficient methods of synthesis, limiting their widespread application. We reasoned that through the combination of photopolymerization of (meth)-acrylates and solid-phase peptide/organic synthesis, we could provide a robust approach for the fabrication of peptide-functionalized hydrogel microarrays for numerous stem cell and tissue engineering applications. To the best of our knowledge, no previous researchers have attempted the combined application of these existing chemistries for the methods outlined in this research [18,28].

Here, we described the development of a platform technology based on light-assisted co-polymerization of poly(ethylene glycol) diacrylates (PEGDA) and methacrylated-peptides to fabricate peptide-functionalized hydrogel microarrays. To this end, we leveraged the high efficiency of solid-phase peptide synthesis and isocyanation chemistry to develop a robust synthetic route for preparing methacrylated-peptides. Due to their high solubility in DMF and high miscibility with low molecular PEGDA, methacrylated-peptides can be effectively incorporated into PEG hydrogels in a ratiometric and homogenous manner. In addition, several parameters were optimized, including the length of the linker between methacrylate functional groups and cell-binding peptide moieties to ensure high accessibility of the peptide functional groups to the cell-surface receptors. The effectiveness of the microarray technology was validated through direct comparison of cell adhesion abilities of highly related RGD peptides: *RGD*, *RGDS*, *RGDS*G and *RGDS*P. To apply the peptide-functionalized hydrogel technology, we constructed a library composed of 12 different RGD peptides to develop synthetic culture substrates for human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), a cell type known for poor adhesion to synthetic substrates [29]. While 6 of the 12 peptides were found through reported literature, bioinformatic screening of ECM proteins led to the identification of 6 unexplored RGD peptides. Notably, 2 out of 6 unexplored RGD peptides showed substantial affinity to hiPSC-CMs. One of them, PMQKM*RGD*VFSP from laminin β4 subunit, was found to have the highest affinity to hiPSC-CMs. With the support of bioinformatic screening, peptide-functionalized hydrogel microarrays are shown here to be a promising strategy to rapidly identify novel biological ligands for the development of functional biomaterials for stem cell and tissue engineering applications.

2. Material and methods

2.1. Materials and instruments

All chemicals used for this study were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Microarray spotting pins (946MP9B) were purchased from Arrayit Corporation (Sunnyvale, CA). A custom designed microarrayer was assembled and produced by BioDot (Irvine, CA). The liquid chromatography-mass spectrometer (LC–MS) system used is Thermo Fisher LCQ Fleet™ Ion Trap Mass Spectrometer.

2.2. Bioinformatics-assisted ECM protein screening

Bioinformatics-assisted ECM protein screening for highly conserved sequences was performed using the following database: UniProt database, which is supported by European Bioinformatics Institute (EMBI-EBI), the SIB Swiss Institute of Bioinformatics, and the Protein Information Resource (PIR). The specific sequence of each ECM protein/ECM protein subunit was collected from mammalian species, including human, mouse, rat, chimpanzee, horse, sheep, rabbit, bovine, guinea pig, cat and dog. The protein alignment was achieved by using the tool of Clustal Omega [30–32] from EMBL-EBI. The algorithm is described by Söding [33]. The highly conserved sequences among different species have been selected out as demonstrated in Fig. S4.

2.3. Monomer preparation and array fabrication

2.3.1. Synthesis and characterization of methacrylated peptides

Peptides used in this work were synthesized by solid phase peptide synthesis (SPPS). The SPPS was conducted using the standard procedure described in Novabiochem peptide synthesis manual. To prepare methyacrylated peptides, 2-isocyanatoethyl methacrylate (3 equivalent (eq) dissolved in DMF) was used to react with the terminal amine group of the peptide chain (1 eq) before they were cleaved from the resin. This solid-phase isocyanation chemistry was first reported by Lee Ayres et al. [34]. All the methacrylated peptides prepared in this study were purified by using a Combiflash® purification system (RediSep Rf) in Reversed Phase format using C18 Columns (Teledyne Isco, Lincoln, NE) running a solvent gradient from 100% H_2O to 100% acetonitrile in 15∼20 min. The peptides were eluted from the column at approximately 70% acetonitrile/30% H_2O. The purified peptides were subsequently characterized by LC–MS.

2.3.2. Microarray fabrication

Methacrylated peptides were dissolved in DMF at predesignated ratios and mixed with PEGDA (containing 1% DMPA