Biomaterials 34 (2013) 3758–3762

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Effect of avidin-like proteins and biotin modification on mesenchymal stem cell adhesion

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ARTICLE INFO

Article history:
Received 1 November 2012
Accepted 24 January 2013
Available online 27 February 2013

Keywords:
Avidin
Streptavidin
Neutravidin
Biotin
Mesenchymal stem cells
Non-specific adhesion

ABSTRACT

The avidin–biotin system is a highly specific reaction that has been used in a wide range of biomedical applications, including surface modification and cell patterning. We systematically examined a number of avidin derivatives as the basis for a simple and cost effective tissue culture polystyrene substrate surface modification for human stem cell culture. Non-specific adhesion between human mesenchymal stem cells and various avidin derivatives, media conditions, and subsequent biotinylation reactions was quantified. We observed significant non-specific cell adhesion to avidin and strepthavidin, indicating that previous observations using this system may be artifactual. Seeding of cells in serum free media, blocking with bovine serum albumin, and the use of the avidin derivative neutravidin were all necessary for elimination of background adhesion. Neutravidin conjugated with biotinylated bsp-RGD(15) peptide provided the most robust cell adhesion, as well as the greatest increase in cell adhesion over background levels.

1. Introduction

The interaction between avidin and biotin is highly specific, and has one of the highest known non-covalent bond strengths, with a dissociation constant of $5^{*}10^{-14}$ M [1]. Due to the strength and specificity of this reaction, the avidin–biotin conjugation system has been used in the biological, biointerface, and sensor communities for a wide range of applications, including: western blots and ELISA assays [2–5]; protein and cell purification from solid substrates [6]; and, affinity chromatography [7]. The avidin/biotin scheme has also been used in a wide range of cell patterning studies, as the ability to create multiple structured layers is advantageous in these schemes. For example, patterns of biotin have been created using photolithographic processes with a novel resist [8], patterns of streptavidin have been created by "scraping" off a protein resistant poly(ethylene glycol) (pEG) layer with a scanning probe [9], and patterns of avidin or streptavidin have been microcontact printed onto polymeric substrates [10,11].

Avidin itself, a protein isolated from avian egg whites, is a tetrameric glycoprotein that can bind up to four biotin molecules, with each subunit binding individually to one biotin. Although avidin has been used in the aforementioned applications, the protein has been implicated in non-specific adsorption to substrates, binding to other biomolecules in bioassays, and adhesion to cell membranes due to avidin's glycosylation and net positive charge [12]. Therefore, a number of derivatives have been developed, including both streptavidin and neutravidin that eliminate the carbohydrate groups from the protein [13]. However, RYG amino acid sequences present on the surface of the streptavidin molecule are thought to structurally mimic the ubiquitous cell adhesive RGD sequence found in many cell adhesion proteins, resulting in mammalian cell adhesion to the protein [14]. Neutravidin was designed to have a near neutral isoelectric point, further minimizing non-specific and electrostatic interactions [15].

Despite the widespread use of avidin, and its derivatives, in the biointerface and biomaterials communities, to date no study has systematically examined the potential non-specific interactions between mammalian cells and various avidin derivatives, and subsequent biotinylation reactions. In this work, we examined peptide-modified biotin as a mimetic for the extracellular matrix proteins that stem cells exploit for adhesion [16]. Two deposition schemes were explored for conjugating peptides to the surface (Fig. 1). In Scheme 1, the avidin (or analog) was simply physisorbed

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http://dx.doi.org/10.1016/j.biomaterials.2013.01.081

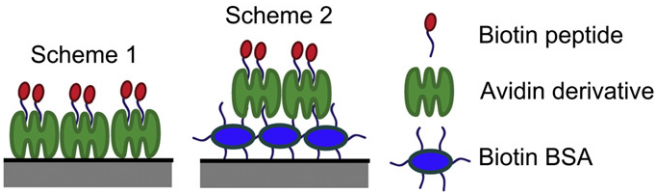

Fig. 1. Avidin/biotin deposition schemes. In Scheme 1, the avidin protein (or derivative) was simply physisorbed to a polystyrene surface before conjugating with a biotin molecule of choice. In Scheme 2, a biotinylated-BSA molecule was first deposited, followed by avidin and a second biotin-linked molecule.

Table 1
Avidin and biotin derivatives tested.

Avidin derivatives	Biotinylated molecules
Avidin	Biotin-RGD
Streptavidin	Biotin-RGE
Neutravidin	Biotin-AG73
Streptavidin-RGD	Biotin-pEG

onto a tissue culture treated polystyrene substrate (TCPS), followed by conjugation with a biotin-grafted molecule of interest. In Scheme 2, the substrate was first blocked with a biotinylated bovine serum albumin (BSA) to separate the avidin molecule from the surface and prevent potential denaturation caused by adsorption. Human mesenchymal stem cell adhesion to standard tissue culture polystyrene surfaces modified using both these schemes, presenting a number of avidin derivatives and biotinylated molecules of interest, was quantified.

2. Methods

2.1. Surface preparation

A number of combinations of albumin blocking, media conditions, and avidin derivatives were deposited onto polystyrene surface in an attempt to minimize non-specific cell adhesion, and are listed in Table 1. Biotinylated-BSA and avidin derived proteins were adsorbed from PBS (pH 7.2) at 0.1 mg/mL for 1 h followed by three rinses with PBS. Biotin-containing molecules were conjugated to the avidin derivatives for 30 min at 0.1 mg/mL in PBS, and rinsed three times. For blocking experiments, the modified surfaces were exposed to a 1% solution of bovine serum albumin (BSA, Sigma) or BSA that had been inactivated for 45 min in a 60 °C water bath (HI-BSA) for 60 min. Surfaces were kept bathed in PBS until cell seeding. Avidin, streptavidin, and neutravidin were all purchased form Invitrogen (Carlsbad, CA). All peptides were purchased from American Peptides (Sunnyvale, CA). Biotin-pEG was purchased from Nanocs (New York, NY). Streptavidin-RGD was graciously donated by Patrick Stayton's lab at the University of Washington [17].

A number of biotin-peptides were studied on the surfaces prepared in this paper, including a 15 amino acid peptide containing the sequence RGD that had been derived from bone sialoprotein (biotin-CGGNGEPRGDTYRAY-NH2, termed biotin-bsp-RGD(15)), and interacts with the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins [18–20]. A peptide sequence termed AG-73 was also tested, consisting of the amino acid sequence biotin-RKRLQVQLSIRT, termed biotin-AG-73 that is believed to interact with the heparin sulfate side chains of the Syndecan-I transmembrane protein, and thought to provide a separate cell adhesive pathway to integrin binding [18,19,21,22].

2.2. Cell culture and quantification

Human mesenchymal stem cells (Lonza, Walkersville, MD) were cultured in MSC growth medium (Lonza) containing 10% serum and 1% gentamicin. For adhesion experiments, cells were allowed to adhere for 4 h onto the surfaces in a 96 well plate at $\sim 10^4$ cells/well (100 μL/well) in hMSC growth media containing serum (Lonza) or serum free DMEM (Gibco/Invitrogen, Carlsbad, CA) depending on experimental conditions. After 4 h, the cells were rinsed once with PBS and frozen for >24 h at −80 °C, followed by quantification of the number of adhered cells using the CyQuant assay (Invitrogen, Carlsbad, CA) using a Gemini Spectramax fluorimeter (Molecular Devices, Menlo Park, CA). Standard curves to calculate cell density based on relative fluorescent units (RFUs) were generated by creating a stock solution of known cell density (counted on a hemocytometer), and performing serial dilutions before applying the same CyQuant assay.

Cells were examined on peptide-modified surfaces to assess morphology using a Nikon T300 in phase contrast mode. For this, cells were plated at 10^4 cells/cm^2 in either a 24 or 48 well plate on the desired surface in serum free media. Cells were allowed to attach for 1 h before being rinsed with fresh media and imaged. The remaining cells were imaged again after 1 d to visualize morphology of spread and proliferating cells.

2.3. Statistics

Each experiment was run in triplicate and analysis of variance (ANOVA) was conducted, and a value of $p < 0.05$ was used to determine significance. Differences among surfaces and media conditions were assessed using Tukey's honestly significant difference (Tukey HSD) post-hoc pairwise comparison.

3. Results & discussion

We initially examined the effect of both serum and heat inactivation of bovine serum albumin (HI-BSA) in a passivating blocking step on hMSC attachment to avidin and avidin/biotin-bsp-RGD(15) surfaces. Samples were constructed as shown in Scheme 1 of Fig. 1:

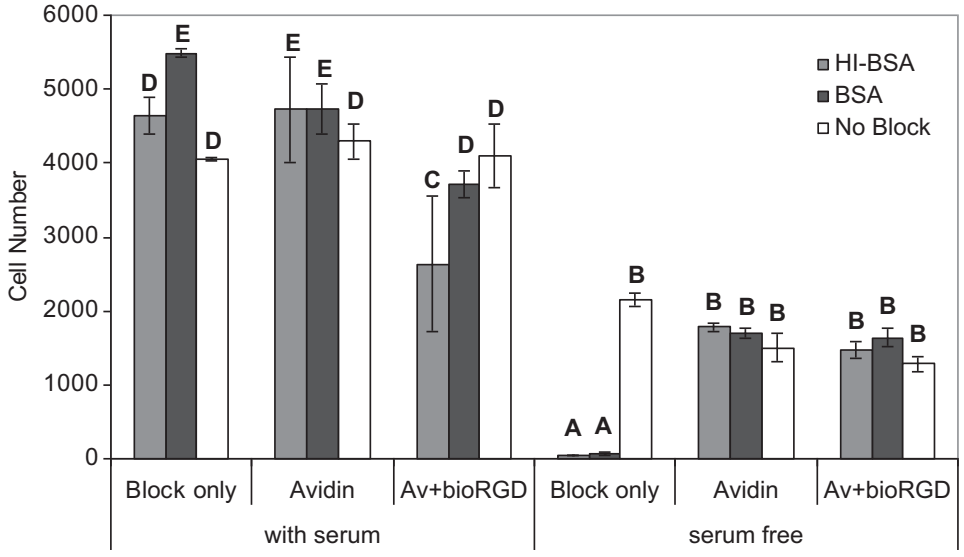

Fig. 2. Effect of heat inactivated BSA (HI-BSA) and serum on human MSC adhesion to avidin adsorbed to TCPS. Samples were prepared as shown in Scheme 1 of Fig. 1. Groups marked with different letters (A–E) are statistically significant ($p < 0.05$); ANOVA with TukeyHSD post-hoc test.