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High-throughput Discovery of Synthetic Surfaces that Support Proliferation of Pluripotent Cells

Ratmir Derda^{†,||}, Samira Musah[†], Brendan P. Orner^{‡,§}, Joseph R. Klim[¶], Lingyin Li[†], and Laura L. Kiessling^{†,‡,*}

[†]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706.

[‡]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706.

[¶]Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI 53706.

Abstract

Synthetic materials that promote the growth or differentiation of cells have advanced the fields of tissue engineering and regenerative medicine. Most functional biomaterials are based on a handful of peptide sequences derived from protein ligands for cell surface receptors. Because few proteins possess short peptide sequences that alone can engage cell surface receptors, the repertoire of receptors that can be targeted with this approach is limited. Materials that bind diverse classes of receptors, however, may be needed to guide cell growth and differentiation. To provide access to new materials that promote cell growth, we utilized phage display to identify novel peptides that bind to the surface of pluripotent cells. Using human embryonal carcinoma (EC) cells as bait, approximately 3×10^4 potential cell-binding phage clones were isolated. The pool was narrowed using an enzyme-linked immunoassay: 370 clones were tested, and seven cell-binding peptides were identified. Of these, six sequences possess EC cell-binding ability. Specifically, when displayed by self-assembled monolayers (SAMs) of alkane thiols on gold they mediate cell adhesion. The corresponding soluble peptides block this adhesion, indicating that the identified peptide sequences are specific. They also are functional. Synthetic surfaces displaying phage-derived peptides support growth of undifferentiated human embryonic stem (ES) cells. When these cells were cultured on SAMs presenting the sequences TVKHRPDALHPQ or LTTAPKLPKVTR in chemically defined media (mTeSR), they express markers of pluripotency at levels similar to those of cells cultured on Matrigel. Our results indicate that this screening strategy is a productive avenue for the generation of materials that control the growth and differentiation of cells.

Introduction

The ability to grow isolated human cells outside of the body (ex vivo) has led to advances in fields ranging from fundamental cell biology to drug discovery. More recently, methods to expand human cells from progenitor and stem cells ex vivo have been developed, and this approach holds great therapeutic promise. Reaping this promise hinges on establishing

* Corresponding Author: Kiessling LL, University of Wisconsin, Department of Chemistry, 1101 University ave., Madison, WI 53706. kiessling@chem.wisc.edu.

^{||}Current Address: Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, 02138

[§]Current Address: Division of Chemistry & Biological Chemistry, School of Physical & Mathematical Sciences, Nanyang Technological University, Singapore 637616.

Supporting Information Available: Experimental procedures, characterization of compounds and supporting information figures S1–S6, supporting information table S1 and a complete reference ²⁵. This material is available free of charge via the Internet at <http://pubs.acs.org>.

conditions that reproducibly support the growth and differentiation of pluripotent cells. As *in vivo*, the *ex vivo* growth and differentiation of cells can be guided by the signals from soluble molecules and insoluble extracellular substrates, including those presented by the extracellular matrix and on the surface of other cells.^{1–3} Historically, products of animal origin, like the soluble components of the serum or the insoluble components of the basement membrane, supply the necessary signals. Still, the heterogeneity of animal-derived components leads to variability in the responses of cultured cells. Moreover, the carryover of pathogens or immunogens from animal products can complicate the use of human cells in therapeutic applications.⁴ The quest for “chemically defined” growth conditions was initiated early in the history of cell culture;⁵ yet even now, the identification of such conditions requires trial-and-error screening of a plethora of formulations. Although the identification of soluble culture media can be performed rapidly via high-throughput screening, methods for the rapid identification of insoluble substrates have been lacking. To facilitate identification of synthetic substrates for cell adhesion and growth, we present a method that unites high-throughput screening for specific ligands with array-based screening of defined ligand-presenting surfaces.

We encountered a need for new approaches when we embarked on a project focused on identifying substrates that support undifferentiated proliferation of human embryonic stem (ES) cells. ES cells are pluripotent—they can give rise to any cell type.⁶ The derivations of human ES cells and the related induced pluripotent stem cells (iPS)^{7, 8} have increased interest in cell therapies. Because human ES and iPS cells have the capacity to expand indefinitely *ex vivo*, they can provide unlimited access to any human cell types for research or therapeutic applications. Reaching this goal requires defined culture conditions for expansion of human ES cells in an undifferentiated state. Human ES cells were initially isolated and propagated in a co-culture with murine embryonic fibroblasts.⁶ Subsequently, growth media in which every soluble component is defined have been developed.^{9–11} Nevertheless, even in chemically defined media, human ES cells are cultured on the insoluble substrate Matrigel, a mixture of murine sarcoma-derived extracellular matrix (ECM). Problems associated with the use of such a xenogeneic matrix can be alleviated by using human-isolated ECM⁹ or recombinant ECM components.^{12–14} As an alternative to top-down simplification of biological matrices, we examined a bottom-up approach. Specifically, we sought to identify chemically defined, synthetic, functional substrates that support ES cell growth.

Our strategy is inspired by natural mechanisms that influence cellular decisions. Specific responses can be elicited by interactions between the ECM and cell-surface receptors. Cell adhesion to the ECM *in vivo* can be mediated by the binding of short peptide motifs within ECM proteins to integrin receptors on the cell surface.^{15–17} This observation has been exploited by a number of research groups who have decorated materials with an integrin-binding Arg-Gly-Asp (RGD) sequence to yield peptide-modified substrates that support cell adhesion and growth.¹⁸ The identification of peptide sequences like RGD has been pivotal in advancing biomaterial research, because of the ease of synthesizing, manipulating and tuning the properties of such materials.¹⁹ Still, only a few adhesive peptide sequences have been identified from natural proteins. We reasoned that the identification of cell growth substrates could be accelerated by the discovery of peptide ligands for cell-surface receptors.

To identify novel ligands for cells, we screened random peptide libraries using phage display. Peptide libraries that contain billions of diverse sequences can be obtained by displaying random peptide sequences on the coat proteins of bacteriophages,²⁰ rendering phage display a powerful means to identify peptides that function as cell surface ligands.^{21–23} To this end, intact cells *in vitro* and *in vivo* have been used as screening targets,^{22, 24} and peptides that recognize receptors on the surface of the target cell types have been identified.^{23, 25} We therefore envisioned that phage-display could be used to find peptides that bind to the ES cell surface and that the resulting sequences could be used to fabricate novel ES cell-binding

substrates. Although not every ES cell-binding material can support ES cell growth,²⁶ we postulated that the active peptide sequences from a phage display screen could be used in conjunction with cell array technology^{27, 28} to yield substrates that support long-term adhesion and proliferation of ES cells in an undifferentiated state.

Although our long-term goal is focused on human ES cells, we postulated that ligands with the requisite properties could be identified using any cell line with a cell-surface receptor repertoire akin to that of human ES cells. Because DNA microarray analyses suggest that gene expression levels are similar for the human embryonal carcinoma (EC) cell line NCCIT and human ES cells,²⁹ we employed the former. Unlike human ES cells,³⁰ NCCIT EC cells rapidly grow in standard tissue culture conditions.³¹ Thus, we used phage display panning with intact NCCIT cells to identify peptide ligands. The identified peptides were converted into insoluble substrates by their attachment to alkanethiols, and these were used to form self-assembled monolayers (SAMs) on gold.^{27, 28} From the materials that resulted, we identified substrates that not only bind NCCIT cells but also support the proliferation of human ES cells.

Results and Discussion

Cell-binding peptides from phage display

Our phage display panning strategy is based on the “biopanning and rapid analysis for selective interactive ligands” (BRASIL) technique.²⁴ Suspensions of live ES or EC cells were used as targets. Previous investigations using phage display to identify cell binding peptides have employed multiple rounds of panning and amplification.^{20, 21, 24} To test this approach for our purposes, we conducted a screen employing three rounds of panning and amplification starting from a commercially available library of M13 phage displaying random 12-mer sequences on the N-terminus of pIII minor coat protein. Before or after each panning step, 48 clones were picked randomly for sequencing to monitor the diversity of the library. Because intact cells present hundreds of different receptors, we expected to identify a large number of cell-binding peptides. In two independent screens, however, the library converged at the third round of screening, and 4–5 peptides dominated the sequence pool. A comparison of the number of unique sequences at each screening step revealed that convergence occurred not at the panning step but rather during amplification (Supporting Information Figure S1 and Table S1). The problem of amplification-induced convergence is exacerbated when cells are used as bait, because the number of potential cell-surface targets is high and therefore the pool of target-binding phage clones in the screen will be diverse. The pool can be narrowed by carrying out a negative selection step using “control cells”²⁴, but for our experiments, there were no appropriate control cell lines. Thus, we developed a funnel screening protocol that provided the means to identify peptides with the requisite cell-binding properties.

In principle, amplification-induced convergence can be eliminated by deleting the amplification step from the screen. This change, however, complicates the isolation of cell-binding phage that act via peptide-specific interactions. Thus we needed a strategy to optimize the screening conditions to maximize the identification of cell-binding phage. To this end, we used wild type (wt) or “environmental” M13 phage, which contain no peptide insert, as reporters for “non-specific” phage (Fig. 1A). Phage that contain peptide inserts bear the *lacZα* reporter gene and thereby give rise to blue plaques (upon infection of bacterial lawn on a solid media containing the chromogenic substrate X-gal). “Environmental” phage, however, give rise to colorless plaques. Because only peptide-bearing phage should bind to cell surface ligands, the ratio of blue to white plaques provides a measure of the specificity of the screen. If the screening conditions are non-selective, wt and library phage will bind similarly, and no change in the blue/white ratio will be observed. If peptide-specific interactions occur, an enrichment of the peptide-containing (“blue”) phage will be detected.

The aforementioned strategy was employed to develop an effective phage panning protocol against live NCCIT cells. A mixture of wt and library phage (1:1 ratio, 10^{11} phage particles each) was incubated with EC cells, and the cells were pelleted by centrifugation to remove the unbound phage. After several washes, the cells were subjected to a more stringent protocol to remove unbound phage—centrifugation in a biphasic aqueous/organic system (a “BRASIL wash”).²⁴ The phage titer was monitored at each step of the screen (Fig. 1B). After 5 rounds of centrifugation-based washes and one “BRASIL wash”, the phage associated with the cell pellet exhibited a 30-fold increase in blue/white ratio (Fig. 1B). The resulting phage-bearing pellet was mixed with EC cells and subjected to another round of incubation and washing. Two consecutive rounds of panning resulted in a 50-fold enrichment in the amount of peptide-bearing phage, and yielded 3×10^4 phage particles. Although the panning procedure led to enrichment of a population of specific phage, many non-specific phage were retained in the pool. To identify the EC-binding phage, we implemented a secondary screen.

Narrowing the pool: Identification of cell-binding phage clones with an enzyme-linked immunosorbent assay

An enzyme-linked immunosorbent assay (ELISA) is a convenient technique for high-throughput detection of phage binding.³² We developed an ELISA using an NCCIT cell suspension as a target, because this assay configuration minimizes false-positive signals that result from the binding of phage to the plate. Cells suspended in 96-well plates were incubated with sub-cloned phage, and a solution of anti-M13 antibody conjugated to horseradish peroxidase (HRP) was added. After the cells were washed via centrifugation, the cell pellet was probed with HRP substrate (Fig. 1C, Supporting Information Figure S6). This cell-suspension ELISA (CS-ELISA) provided the means to probe rapidly hundreds of phage clones. We tested a sub-population of $\sim 3 \times 10^4$ clones (1.3%) isolated by EC cell panning (Fig. 1B), and from 370 clones tested, 7 cell-binding phage clones were identified (Fig. 1D). These data indicate that a combination of amplification-free panning and high-throughput CS-ELISA can readily yield hundreds of potential cell-binding peptide sequences.

Evaluating cell-binding properties of the phage-derived peptides with surface arrays

We sought to determine whether the identified peptides can be employed to generate materials that support cell adhesion and growth. To this end, we appended the identified cell-binding peptides to synthetic surfaces and evaluated their ability to support adhesion of NCCIT cells. The immobilization approach we employed has several key features. First we avoided immobilization techniques that yield random orientation of peptides on the surface (e.g. non-specific adsorption), because they can afford non-productive orientations of the peptide that decrease binding. Second, we noted that the attachment of peptides to the surface through their N-termini also might diminish binding, because phage-presented peptides possess N-termini that can contribute to target recognition.³³ Accordingly, we linked the identified peptides to defined synthetic surfaces through their C-termini. Finally, the surface distribution of the peptides can have dramatic effects on cell binding;^{34, 35} hence, we evaluated surfaces presenting different peptide densities.

The arrays we employed consist of self-assembled monolayers (SAMs) of alkanethiols (AT) on gold. These surfaces are well-defined because the orientation and density of the surface-presented compounds can be easily controlled.^{27, 36} To create the desired display, we synthesized peptides with free N-termini and a C-terminal AT substituent.³⁷ When a solution of AT-peptide is spotted onto the gold surface (Fig. 2B), an oriented C-terminal surface display of peptides is formed. Using a two-step fabrication strategy (Fig. 2A), arrays presenting different peptides at different densities were generated and tested for their ability to support adhesion of NCCIT cells. We employed serum-free media for cell-binding studies to eliminate the non-specific adsorption of cell-adhesive proteins. To minimize the influence of cell-

secreted proteins, we used short-term cell adhesion (1 hour). Of the seven phage-derived peptides identified, six supported cell binding. The SAMs presenting LTTAPKLPKVTR were especially effective, as they supported cell adhesion over the broadest range of densities (Fig. 3A, B).

SAMs displaying phage-derived peptides support adhesion of EC cells through peptide-specific interactions

We sought to determine whether cell-binding is mediated by specific interactions with a peptide presented by the surface. Evidence for specificity can be obtained by assessing whether a soluble inhibitor blocks cell binding to the surface.³⁸ If the binding is due to non-specific effects, a soluble competitor will have little effect. We therefore tested whether phage displaying a peptide sequence of interest inhibit cell adhesion to the peptide-presenting monolayer. The competition experiments were conducted using concentrations at which wt phage had no effect on cell binding (Supporting Information Figure S2). Under these conditions, the addition of VTSRTIIPQGS A-presenting phage diminished cell adhesion to the VTSRTIIPQGS A-substituted surface.

We also assessed the abilities of soluble peptides to block cell adhesion. These competition studies were carried out using soluble peptide concentrations that have no non-specific effects (e.g., no general toxicity, see: Supporting Information Figure S2). The data indicate that soluble LTTAPKLPKVTR can compete with cells for a surface presenting the cognate peptide sequence (Fig. 3C, D). In contrast, another phage-derived peptide sequence (TVKHRPDALHPQ) had little effect. These findings indicate that adhesion to the LTTAPKLPKVTR-modified surface is specific. Moreover, the competition data suggest that LTTAPKLPKVTR and TVKHRPDALHPQ interact with different cell surface targets. Thus, our phage display screen can yield surfaces that support EC cell adhesion via independent mechanisms.

Human ES cells proliferate on LTTAPKLPKVTR- and TVKHRPDALHPQ-substituted monolayers

Given the aforementioned similarity of the ES and EC cell gene expression profiles,²⁹ many receptors on the EC cell-surface should be present on human ES cells. We therefore postulated that the sequences identified using EC cells could be used to generate synthetic substrates for human ES cell binding and proliferation. The ability of SAMs to support short-term cell adhesion (Fig. 3), however, does not predict their performance in long-term growth assay. Many substrates that engage in short-term binding of human ES cells fail to support their proliferation over longer time periods.^{9, 12, 13, 39} To determine whether any of the peptide-displaying SAMs can facilitate human ES cell proliferation, we cultured human ES cells on these surfaces for multiple days, using conditions that are stringent and chemically defined. Specifically, we used the media mTeSR-19,⁴⁰ supplemented with an inhibitor of Rho-associated kinase (ROCK inhibitor, Y-27632),⁴¹ The latter was added because it can improve human ES cell culture by decreasing apoptosis of single hES cells.⁴² To minimize cell adhesion resulting from adsorbed cell-secreted factors, we avoided using a high ES cell density (e.g., 10^6 cells/mL) and employed the conditions (4×10^4 cells/mL) used in standard human ES cell culture.

Human ES cells (H9) were proliferated on the peptide-displaying SAMs, and the results were compared to those of cells cultured on Matrigel, the standard substrate employed in ES cell culture.³⁰ Of the six peptide-modified SAMs that support NCCIT cell adhesion, five were effective at mediating human ES cell adhesion. These results confirm our initial hypothesis that human EC cells could be used in screens to identify ligands for human ES cells. Three of the active surfaces, those presenting QLGTQPNRSTYA, SRYITTMNTEQV or

VTSRTIIPQGS (data not shown) could promote short-term human ES cell adhesion (Supporting Information Figure S3A), but failed to sustain binding and proliferation over 5 days (Fig. 4). In contrast, SAMs derived from the other three peptide sequences (LSTIGMPKLLLA, LTTAPKLPKVTR and TVKHRPDALHPQ) could promote human ES proliferation over the longer time period (Fig. 4). Staining for two markers of pluripotency, Oct4 and SSEA-4,³⁰ revealed that most cells on the LSTIGMPKLLLA surface had differentiated. In contrast, a majority of the cells proliferated on substrates presenting either LTTAPKLPKVTR or TVKHRPDALHPQ retained the features of pluripotent ES cells (Fig. 4B,, 5A and Supporting Information Fig. S3B, C).

These successes led us to evaluate the utility of the surfaces over longer time periods. Specifically, human ES cells were proliferated on LTTAPKLPKVTR- and TVKHRPDALHPQ-substituted monolayers in defined (TeSR-ROCK) media for 20 days (3 passages). The results were compared to those propagated using Matrigel under either the same conditions or with a differentiation-inducing medium.³⁹ Flow cytometry analysis after twenty days of culture indicated that cells grown on the LTTAPKLPKVTR or TVKHRPDALHPQ substrates maintained a high level of Oct4 expression. Indeed, it was similar to that of cells cultured on the Matrigel substrate in TeSR-ROCK media (Fig 5B) and significantly higher than those cultured in conditions that induce differentiation. These results indicate that the peptide-modified surfaces can maintain cells in an undifferentiated state even after several weeks of culture.

Because the culture of human ES cells in sub-optimal conditions can lead to the accumulation of chromosomal abnormalities,⁴³ we examined the karyotype of the cells proliferated on peptide-modified surfaces (Supporting Information Figure S4). After 25 days of culture, non-clonal abnormalities were detected in a small number of cells from the TVKHRPDALHPQ-presenting monolayer (2 out of 40), but no clonal abnormalities were observed. No karyotypic abnormalities were detected in cells proliferated on the LTTAPKLPKVTR-displaying monolayer even after 49 days of culture. For both sequences, longer term growth is necessary to confirm the karyotypic stability of cells under these conditions. Nevertheless, the data suggested that these novel peptide-presenting surfaces can support the undifferentiated growth of human ES cells.

Human ES cells bind to LTTAPKLPKVTR- and TVKHRPDALHPQ-substituted SAMs and Matrigel-coated surfaces via different receptors

As a step toward dissecting the mechanisms by which the active phage-derived peptides function, we tested the involvement of receptors known to mediate cell adhesion. The receptor for these peptides is not obvious, as a BLAST search indicated that they have no significant sequence similarities to known extracellular proteins. We therefore considered potential receptor candidates on the basis of proteins known to mediate cell adhesion. The interaction of human ES cells with surfaces coated by ECM-proteins is mediated largely by cell-surface integrins.^{12, 39} Alternatively, cadherins are another protein family that can be critical for ES cell adhesion.⁴⁴ Both of these receptor classes require divalent metal cations (Ca^{2+} , Mn^{2+}),⁴⁵ and their action is obliterated by the metal chelator EDTA. Other cell-surface entities known to be important for cell-ECM interactions are anionic glycosaminoglycans (e.g. heparan sulfate proteoglycan (HSPG)).⁴⁶ Many glycosaminoglycan-dependent, including HSPG-dependent interactions can be blocked by soluble heparin.⁴⁷ We tested the effect of EDTA and heparin on human ES adhesion to different substrates. As a positive control for HSPG-dependent interactions, we utilized adhesion of hES cells to poly-L-lysine coated surfaces, as this binding interaction is mediated by Coulombic attraction: EDTA had no effect, but heparin inhibited human ES cell adhesion. Interestingly, cell adhesion to Matrigel also was reduced by 45% in the presence of soluble heparin. Moreover, as expected, the addition of EDTA reduced adhesion

to Matrigel by >80%, an observation that indicates that human ES cell-Matrigel interactions are driven primarily by integrin interactions.³⁹ In contrast, the adhesion of human ES cells to LTTAPKLPKVTR-substituted monolayer was reduced modestly (~30%) in the presence of EDTA or heparin. Even more strikingly, neither inhibitor had an effect on the adhesion of human ES cells to TVKHRPDALHPQ-displaying monolayers (Fig 5C). These results suggest that phage-derived peptides, unlike Matrigel³⁹ or vitronectin,¹² bind neither integrin receptors nor proteoglycans. We therefore conclude that the interaction of peptides with human ES cells occurs through different types of receptors. As a consequence, the strategy that we have outlined affords entities that can illuminate new molecular mechanisms that can regulate the adhesion and self-renewal of pluripotent cells.

Conclusions

Our results demonstrate that a bottom-up approach can be used for the *de novo* discovery of synthetic cell growth substrates. In this way, fully defined, synthetic substrates may replace complex biological matrices of animal origin. Indeed, the ability of our peptide-displaying SAM to serve as a substrate that can support undifferentiated proliferation of human ES cells highlights the promise and the utility of our method. Although Matrigel and other matrices that support stable long-term ES cell culture function through cell adhesion receptors, like the integrins, neither LTTAPKLPKVTR nor TVKHRPDALHPQ act through these receptors. These findings highlight that our screening strategy can yield new avenues for investigating the receptors and mechanisms that govern cell growth and differentiation.¹⁵ The strategy we have described is applicable to discovering new cell surface receptor–ligand pairs for a wide variety of cell types, including hematopoietic stem cells, induced-pluripotent stem cells, and cancer stem cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) A 1:1 mixture of peptide-presenting (library) and peptide-free (wt) phage was used to optimize the screen for NCCIT-binding phage clones. (B) The amount of peptide-bearing (“blue”) and wt (“white”) phage was monitored at each panning and washing step (see Supporting Information for details). Through multiple panning steps, a steady increase in the blue/white ratio is observed. (C) The phage population that remained associated with cells after all washing steps (denoted as “R2 BR pellet” in B) was sub-cloned; the clones were amplified and tested using the cell suspension ELISA (CS-ELISA) to identify those that bind to NCCIT cells. (D) Testing of 92 phage clones (sub-library 1, left scale) and 276 phage clones (sub-library 2, right scale) using the CS-ELISA yielded 2 and 5 clones, respectively, that bind to NCCIT cells significantly better than wt phage. The results are averages from two independent measurements. Error bars indicate one standard deviation.

Figure 2.

(A) Method for fabrication of surface arrays of peptides.²⁷ SAMs composed of perfluorinated alkanethiol on gold were photopatterned. Solutions that contain peptide-AT, glucamine-AT or mixtures of the two were spotted onto the exposed gold regions. The ratio of peptide-AT and non-cell-adhesive glucamine-AT in solution was altered to vary the surface concentration of peptide. (B) Route for the synthesis of the peptide-AT starting from the AT resin.³⁷ (C) Structures of the glucamine-AT and perfluoro-AT.²⁷

Figure 3.

Arrays were prepared by spotting solutions containing mixtures of peptide-AT and glucamine-AT (1 mM total concentration) onto patterned perfluoro-AT SAM (see Figure 2). The percentages are derived from the mole fraction of the peptide-AT in the solution from which the array element was generated. NCCIT cells were incubated with arrays for 1 h in serum-free media. The cells were then fixed with formaldehyde and stained with Hoechst 33342 to visualize cell nuclei. (B) The number of cells on each array element was determined by counting nuclei using ImageJ software. Data are average of 3–6 measurements from three independent experiments; error bars indicate one standard deviation. (C) The short-term cell adhesion of NCCIT cells to monolayers presenting LTTAPKLPKVTR was inhibited with soluble LTTAPKLPKVTR peptide. Another phage-derived peptide that also supports cell adhesion, TVKHRPDALHPQ, had little effect. (D) The ability of the soluble peptides to inhibit binding was quantified using ImageJ software. Data presented are the average of six measurements; error bars are one standard deviation; (*) $p < 0.05$ and (**) $p < 0.01$ as determined by two-tailed unequal variance t-test. (A) and (B) represent mosaic images obtained with a fluorescent microscope with 6.4x objective. An inverted image of the blue-fluorescent channel is presented.

Figure 4.

(A) Monolayers that present phage-derived peptides were tested for their ability to support prolonged growth of human ES cells in a defined medium. Human ES cells (line H9) were plated in mTeSR1 media in the presence of ROCK inhibitor (Y-27632) atop SAMs displaying the indicated peptide sequence. Cells were allowed to proliferate for five days; the media was exchanged daily. At day 5, the cells were fixed and stained with anti-Oct4 goat IgG and anti-SSEA-4 mouse IgG, followed by Alexa 488 anti-goat-IgG and Alexa 555 anti-mouse-IgG, and Hoechst 33342. Inverted images of each fluorescent channel (Hoechst, Oct4, SSEA-4) are presented. Arrows (Hoechst, second row) point to groups of cells (B) Quantification reveals that two of five peptides support the proliferation of undifferentiated human ES cells. SAMs

presenting the sequence LSTIGMPKLLA support cell growth but induce differentiation. The cell count in each fluorescent channel was estimated by integrating the area of the fluorescent signal. The acquisition time and signal threshold used for the quantification of each fluorescent channel were set to identical levels for all images. For additional images see Supporting Information Figure S3.

Figure 5.

Images depicting human ES cells proliferating on SAMs that present the peptide sequences LTTAPKLPKVTR or TVKHRPDALHPQ (abbreviated as “LT...R” and “TV...Q”). (A) Human ES cells were plated onto the SAMs in mTeSR1 media supplemented with 5 μM Y27632 ROCK-1 inhibitor (mTeSR-ROCK). On day 5, the cells were fixed and stained with anti-Oct4 and anti-SSEA4 antibody to visualize markers associated with pluripotency. Scale bar 500 μm . (B) Cells were cultured on peptide-substituted SAMs in mTeSR-ROCK medium for 20 days (three passages). The cells were then harvested and stained with anti-Oct4 PE-conjugate, and their staining was compared to cells proliferated on Matrigel (positive control) and those proliferated on Matrigel in a differentiation-inducing medium (negative control).³⁹ (C) Inhibition of human ES cell adhesion: A suspension of human ES cells containing 10 mM EDTA or 5 mg/mL heparin was added to wells coated with Matrigel, poly-lysine, or wells with gold-coated peptide-SAM substrates. Cells were allowed to bind for 1 h, and the number of adherent cells was analyzed using CellTiter-Glo. Neither heparin nor EDTA treatment exhibits significant effect on short-term human ES cell adhesion to peptide SAM-presenting substrates.