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Opinion

Discovering Cell-Adhesion Peptides in Tissue Engineering: Beyond RGD

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As an alternative to natural extracellular matrix (ECM) macromolecules, cell-adhesion peptides (CAPs) have had tremendous impact on the design of cell culture platforms, implants, and wound dressings. However, only a handful of CAPs have been utilized. The discrepancy in ECM composition strongly affects cell behavior, so it is paramount to reproduce such differences in synthetic systems. This Opinion article presents strategies inspired from high-throughput screening techniques implemented in drug discovery to exploit the potential of a growing CAP library. These strategies are expected to promote the use of a broader spectrum of CAPs, which in turn could lead to improved cell culture models, implants, and wound dressings.

Highlights

Hydrogel materials with mechanical and biological properties mimicking the ECM are used to organize different cell types into functional tissue. As an alternative to animal-derived ECM proteins, CAPs such as the AA sequence RGD can control the adhesion of cells onto synthetic materials.

Synthetic biocompatible hydrogels do not present specific CAPs that can precisely control cell fate and function. Quick and high-yielding conjugation reactions can link biological signals to synthetic hydrogels in robotized liquid handling systems under physiological conditions.

Automatically fabricated artificial ECM can rapidly create and test cell microenvironments. These platforms can decipher the role of RGD-alternative CAPs, more quickly identify CAPs to control cell function, and extend our control of cell adhesion on synthetic materials.

Cell-Adhesion Peptides in Tissue Engineering

Cell-adhesion peptides (**CAPs**; see Glossary) are short amino acid (AA) sequences that are the minimal motif required to specifically bind to a cell receptor responsible for the cell adhesion. In the past decades, several CAPs have been utilized in synthetic cell culture substrates to recapitulate the cell-binding properties of expensive animal-based macromolecules of the **extracellular matrix** (**ECM**; Box 1). Several types of artificial substrates, such as implants [1], scaffolds [2], fibers [3], and hydrogels [4], have been conjugated with CAPs. These functionalized synthetic systems presenting CAPs have been shown to improve tissue integration of titanium implants [5], induce cell spreading in 3D cell culture [6], and reduce scar formation [7]. While CAPs have allowed tremendous advances in biomedical materials, a search on three databases (PubMed, Scopus, and Web of Science) for articles published between 1970 and early 2018 on CAPs used to functionalize scaffolds, hydrogels, implants, and fibers reveals a paucity in the number of investigated peptides (see Figure S1 in the supplemental information online). The majority (89%) of the published studies used the RGD AA sequence, which targets the integrin receptor. The second and third most reported, IKVAV and YIGSR, both isolated from the laminin sequence, represented only 6% and 4% of the publications, respectively. Other marginal CAPs such as DGEA, PHRSN, and PRARI represented less than 1% of the literature. The over-representation of RGD in biomaterial composition is staggering. Several factors can explain the lack of variety in the CAPs used in **tissue engineering**: these CAPs work (they induce cell spreading and adhesion), they are soluble in aqueous media, they are short and easy to manufacture at a high purity in automated peptide synthesizers, and they are well characterized. By contrast, implementing new CAPs can be a challenging task where many parameters need to be optimized including peptide solubility, concentration, stability, and chemical binding to a medical device or a cell culture **substrate**.

This Opinion paper presents several CAPs yet to be implemented on cell culture substrates and proposes different strategies to efficiently trial these CAPs for their use in medical devices, implants, or 3D cell culture applications.

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Untapped Knowledge

Engineering tissue models or medical devices requires a comprehensive understanding of the interactions between cells and their environment. The discovery of fibronectin and its role in cell adhesion has paved the way for a greater appreciation of the role played by the ECM macromolecules in cell adhesion [8]. Subsequently, the minimal binding domain of fibronectin to the integrin receptor, RGD, was isolated. Ever since, this short peptide has been abundantly utilized to induce cell adhesion in synthetic systems. However, the ECM includes other proteins and polysaccharides in different ratios that depend on the type of tissue. Therefore, to accurately replicate the complexity of the ECM, more than one CAP with nonspecific integrin binding should be used. Beyond the well-characterized and utilized RGD, IKVAV, and YIGSR peptides, many CAPs derived from ECM proteins have been identified, and their receptor-binding specificity has been described (Table 1). These CAPs can mimic the cell adhesion of different ECM macromolecules by explicitly targeting a cell receptor. Applying these CAPs alone or as a combination would provide new avenues for designing complex ECM replicates able to target diverse cell receptors. Nonetheless, CAP sequences have to be chosen carefully as their physico-chemical properties might make them challenging to implement in tissue engineering applications.

One considerable concern for the functionalization of synthetic systems, and particularly hydrogels, is the solubility of the peptide sequence. For instance, aqueous-insoluble peptides might be difficult to conjugate to hydrogels. Therefore, estimating the peptides' properties could help to select suitable CAPs for any particular application. One way to predict the aqueous solubility of a peptide is by assessing the chemical properties of the AAs in the peptide sequence. AAs with aliphatic and aromatic hydrophobic side chains will lower the water solubility, while acidic and basic groups like histidine and glutamic acid will have the opposite effect [9]. Likewise, charges on the peptide will affect the solubility and might require utilizing acidic or basic aqueous media for the peptide solubilization [10]. Furthermore, cysteine residues in the sequence can enhance its stability against proteases due to spontaneous formation of disulfide bonds between cysteine residues [11]. To assist in the selection and utilization of these CAPs, the physicochemical properties of the AAs were classified and coded for their hydrophobicity, charges, and polarity (Table 1). This classification helps to rapidly identify CAPs that might be difficult to solubilize, such as elastin CAPs mainly composed of hydrophobic AAs [12,13]. Therefore, taking the peptide's physicochemical properties into consideration could help to generate efficient CAPs libraries and identify compatible CAP combinations.

For biomaterial design, CAPs can be used alone or as a combination of several CAPs, each uniquely binding to one cell receptor [14]. Such combinations can open new avenues for precisely controlling cell function and identifying synergistic effects across CAPs as demonstrated with RGD and YIGSR for regenerating the sciatic nerve [15]. In another example, seven CAPs immobilized on a hydrogel were tested, both alone and as a combination, for the encapsulation of MIN6 mouse insulin producing cells, which revealed that a specific single CAP or combinations of CAPs could increase insulin production [16]. Going beyond integrin and laminin receptors targeting could lead to new ways of controlling cell fate and function. CAPs explicitly targeting one integrin receptor, heparin receptor, or CD157 (a leukocyte surface receptor) [17] could lead to new *in vitro* tissue models.

Although incorporating new CAPs in tissue engineering or medical device design is an uncertain and challenging task, low-risk strategies to efficiently identify the appropriate CAP or

Glossary

3D bioprinting: additive manufacturing that deposits cells suspended in a hydrogel for the fabrication of 3D biological structures.
4D cell culture: cell culture in 3D tissue models in which the time is considered as a fourth dimension. For instance, cell behavior can be tracked over time.
Bioink: a suspension of cell in a cytocompatible polymeric matrix that is used for 3D bioprinting.
Cell-adhesion peptides (CAPs): peptide sequences derived from ECM macromolecular proteins involved in the cell-adhesion process through cell receptor binding.
Drug discovery: the process of establishing new drugs, which can start with *in silico* drug design or HTS of molecule libraries on cell cultures. Identified candidate molecules are then processed into drugs for further testing and if successful moved to clinical trials.
Extracellular matrix (ECM): the molecules excreted by cells forming the cell environment and providing support for cells to build tissues and organs.
High-throughput screening (HTS): a robot-assisted method that can test hundreds of thousands of molecules on tissue models to identify future drugs.
Signaling pathway: a cascade of chemical reactions that occurs in a cell when a molecule or CAP attaches to a receptor on the cell membrane.
Substrate: a biocompatible material that can be used for cell culture. It can be planar (2D), have a hemispherical shape (2.5D), or a suspension of cells in the material (3D).
Tissue engineering: design and fabrication of living replacement devices for surgical reconstruction and transplantation.
Tissue replicate: cell organized in a hydrogel reproducing the *in vivo* structure and function of a natural tissue.

Box 1. Engineering Synthetic Cell Microenvironments

The natural cell microenvironment is composed of a variety of proteins and polysaccharide macromolecules. In addition to providing mechanical support, these macromolecules link to the cells through receptors located on the cell membrane (Figure IA). Receptors, such as integrin, participate in connecting the cell's cytoskeleton to the ECM macromolecules. These connections allow the cell to migrate, differentiate, and organize. To recreate the natural cell environment *in vitro*, synthetic substrates are used to mimic the mechanical properties of the natural ECM (Figure IB). Biocompatible substrates utilized for cell cultures such as alginate, agarose, or PEG lack the specific adhesive motifs allowing for the precise control of cell fate and function. As an alternative to natural proteins, short peptides that specifically target cell receptors can be used to create a biological link between the synthetic substrate and the cells. These short peptides, so-called CAPs, can indicate the composition of the environment to the cell. The CAPs are immobilized on the hydrogel-forming polymer by using optimized coupling chemical reactions. In parallel, careful selection of the polymers and their processing into a cell culture substrate can match the cell microenvironment mechanical properties to the properties of natural tissues [4].

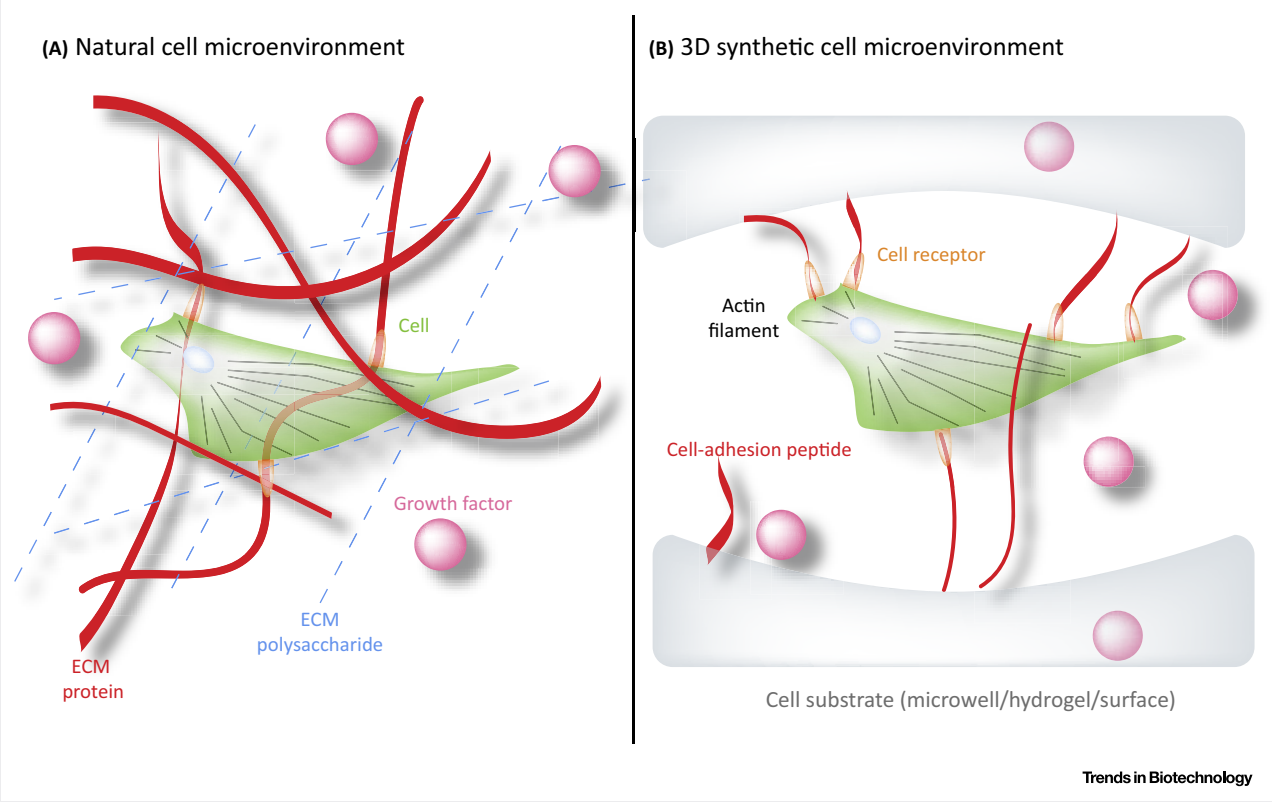

Figure I. Comparison between Natural Tissue and Synthetic Cell Microenvironment. (A) In the native extracellular matrix, cells attach through cell receptors to polysaccharides and proteins. (B) 3D synthetic cell microenvironment made of a substrate with defined mechanical properties functionalized with peptide sequences able to bind to cell receptors. Addition of soluble biological signals, like growth factors, embedded in the artificial matrix can further assist reproducing the original tissue. Abbreviation: ECM, extracellular matrix.

combinations of CAPs for a specific application can be implemented through screening methodologies.

Screening Cell-Adhesion Peptides

Manufacturing **tissue replicates** consists of organizing cells of a specific tissue in a synthetic cell microenvironment that reproduces the main characteristics of ECM, namely, mechanical support, soluble growth factors, and cell–ECM interactions. The optimal combination of these essential ECM features allows control over the fate, organization, and function of cells to build up functional tissues. For example, the mechanical properties of the cell culture substrate in combination with variable CAP concentrations can be used to regulate the fate of

Table 1. Physicochemical Properties of Cell-Adhesion Peptide Sequences Isolated from ECM Macromolecules (Proteins) Binding to Specific Cell Receptors[a,b]

Protein	Sequence	Receptor	FC	IEP	Refs
COL I	*GFOG***ER**	$\alpha_1\beta_1$, $\alpha_2\beta_1$	0	6.7	[43]
	D*G***E***A*	$\alpha_2\beta_1$	–2	3.6	[44]
LAM	*Y**IGS***R**	$\alpha_4\beta_1$, 67 kDa, 38 kDa, 36 kDa	1	9.3	[45,46]
	*Y**IGS***R** (cyclic)	67 kDa	1	9.3	[47]
	*S**IKVAV*	$\alpha_3\beta_1$, $\alpha_6\beta_1$	1	9.7	[48]
	*/**KVAV*	$\alpha_3\beta_1$, 110 kDa, 67 kDa, 45 kDa, 32 kDa	1	9.7	[49–51]
	*/**KLLI*	$\alpha_3\beta_1$	1	9.7	[52]
	*L**RGDN*	$\alpha_v\beta_1$	0	6.2	[53]
	*S**NNNR*	$\alpha_6\beta_1$	1	10.6	[54]
LAMγ1	*L**RE*	–	0	6.3	[55]
	*P**DGSR*	–	0	6.2	[47]
	*GTFAL**RGDNGQ*	VLA-6	0	6.1	[52]
	*CFAL**RGDNP*		0	6.2	[52]
	*NPWHS/Y/T**RFG*	$\alpha_6\beta_1$	1.1	9.3	[56]
	*TWY**K/AFQRNRK*		4	11.6	[56]
	***K**AF**D**/TYVRL**K**F*	$\alpha_5\beta_1$, $\alpha_v\beta_3$	2	10.2	[57]
	LGTIPG	67 kDa	0	6.0	[58]
FN	*G**RGDS*	$\alpha_v\beta_3$, $\alpha_v\beta_5$	0	6.2	[59]
	*P**KRGDL*	$\alpha_v\beta_5$, $\alpha_v\beta_1$	1	9.7	[59]
	*NG**RAHA*	$\alpha_5\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$	1	10.5	[59]
	*GAC**RGDC**LGA* (cyclic)		0	6.0	[59]
	*/**DAPS*	$\alpha_4\beta_1$	–1	3.7	[60]
	***RED**V*	$\alpha_4\beta_1$	–1	4.2	[61]
	*PHS**RN*	$\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$	1	10.5	[62]
	***K**QAGD**V*	$\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$	0	6.2	[63]
	*L**DV*	$\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_4\beta_P$	–1	3.7	[64,65]
	*WQPP**RAR/*	$\alpha_4\beta_1$	2	12.5	[66]
	*SPP**RR**AR**V*	Heparin	3	12.8	[67]
	*LIG**RKK*	Heparin	3	11.8	[68]
	*IW**K**HK**G**RD**V**IL**KKD**V**R**F**YC*	CD157	4	10.4	[69]
	***K**L**D**APT* (FN5)	$\alpha_4\beta_7$, $\alpha_4\beta_1$	0	6.2	[70]
	*P**RAR/* (FN12–14)	$\alpha_4\beta_1$	2	12.5	[71]
VTN	*C**KKQR**F**R**H**RN**RKG*	CD157, Heparin, $\alpha_v\beta_5$	7	12.5	[72]
OPN	***KRSR***	Heparin	3	12.5	[73]
	*F**H**RR/**K**A*	Heparin	3	12.5	[74]
	*CGGNG**EP**RGD**T**Y**R**AY*	$\alpha_5\beta_3$, $\alpha_2\beta_1$	0	6.2	[75]
	*SVVYGL**R*	$\alpha_4\beta_1$	1	9.3	[76]
	***E**LV**T**D**F**PT**D**LPA**T*	$\alpha_4\beta_1$	–3	3.4	[76]
ELN	*VPGIG*	–	0	6.0	[77]

Table 1. (continued)

Protein	Sequence	Receptor	FC	IEP	Refs
	VGVAPG	67 kDa	0	6.0	[78]
COL IV	*M*NYYSNS	$\alpha_v\beta_3$	0	6.0	[79]
	CNYYSNS		0	6.0	[79]
THBS1	CSVTCG	Heparin	0	6.0	[80]
	*G***R***G***D***AC*	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$	0	6.2	[81]
	*FQGVLQNV***R***FVF*	$\alpha_3\beta_1$	1	10.6	[82]
	*A***E***L***D***VP*	$\alpha_4\beta_1$	–2	3.6	[83]
	*VAL***DE***P*		–2	3.6	[83]
NID 1	*GF***R***G***D***GQ*	–	0	6.2	[84]
	*SIGF***R***G***D***GQTC*	Leukocyte response integrin (LRI)	0	6.2	[85]

[a]Italicized letters indicate hydrophobic, underlined indicate polar uncharged, bold indicate polar charged, and normal font indicate cysteine.
[b]Abbreviations: COL I, collagen I; COL IV, collagen IV; ELN, elastin; FC, formal charge at pH 7; FN1, fibronectin; IEP, isoelectric point; LAM, laminin; LAMγ1, laminin γ1; NID1, nidogen-1, closed at the * interface; OPN, osteopontin; THBS1, thrombospondin; VTN, vitronectin.

mesenchymal stem cells [18]. To study cell–CAP interactions, CAPs are covalently bound to the cell culture substrate. Functionalizing 2D or 3D synthetic substrates with CAPs requires the use of coupling chemistries, such as carboxylic acid activation for its reaction with the N terminal of the peptides. As an example, the fibronectin RGD peptide motif was immobilized on agarose polysaccharides chemically modified to bear carboxylic acid functional groups available for peptide coupling. This system was used to induce the 3D luminal organization of endothelial cells [4]. Similarly, alginate, which has native carboxylic acid groups, can be directly functionalized with different CAPs [19]. Alternatively, functional groups can be directly introduced into synthetic polymers such as poly(ethylene glycol) (PEG), allowing for the direct conjugation of CAPs to engineer cell microenvironments that direct epithelial tubulogenesis [20]. Because of the broad parameters to be tested, identifying the optimal combination of mechanical properties, growth factors, CAPs, and cells is a complex task. Therefore, methods for rapidly manufacturing a large number of varied cell microenvironments could help to accelerate the identification of CAPs, or combinations of CAPs, relevant for a specific cell type.

Inspired by **drug discovery** methods, a **high-throughput screening (HTS)** approach can be applied to the fabrication and identification of intricate cell microenvironments. At the beginning of the drug development process, a library of molecules is screened for their activity on models of healthy or diseased tissue. This optimized screening process can be scaled up to test hundreds of thousands of molecules per day in automated systems. The uptake of this research paradigm has had a tremendous impact on drug development with between 20% and 30% of drugs in clinical development being identified through HTS [21].

Recent developments in chemistry and materials science have afforded materials that can be processed and functionalized in automated systems. As such, liquid handling robots that can fabricate a variety of cell culture substrates are particularly well suited for the HTS of the cell environment (Figure 1). One of the best examples of the application of these systems is the utilization of surface-activated glass slides and tissue culture plastic that can be covalently linked with ECM macromolecules to create microarrays of the functionalized surfaces [22]. Such an approach translated to peptides could help to identify CAPs for the functionalization of

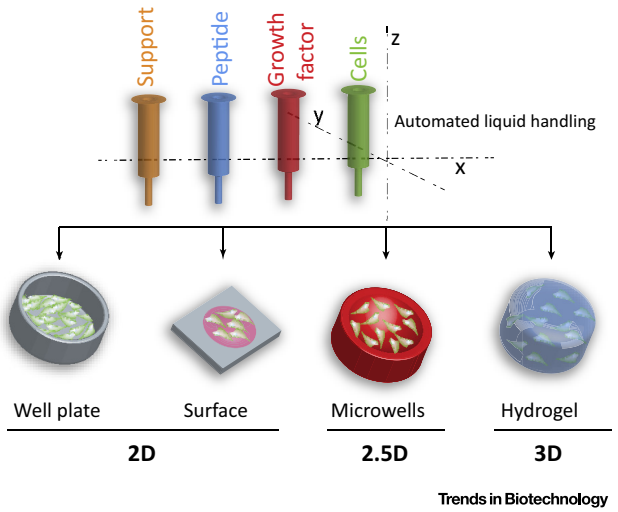

Figure 1. Proposed Platforms for the Screening of Cell-Adhesion Peptides, Allowing for the Assembly of Cells, Substrate, Peptides, and Growth Factors by Automated Liquid Handling Robots.

orthopedic implants made of polymers or metal alloys. Nevertheless, screening platforms are not only limited to 2D geometries. Microwell platforms ('2.5D' geometry) can also be manufactured and functionalized in a single automated step. As an example, poly(dimethylsiloxane) microwells with localized reactive anchors [23] and growth factor-functionalized collagen microwells were both made with a liquid handling robot [24]. Further iterations of these systems can lead to multi-CAP-functionalized microwell platforms for screening cell–CAP interactions.

Extending on the microwell concept is 3D cell culture. The use of rapid aqueous-compatible addition reactions based on thiol-ene [25], Diels–Alder [26], or Huisgen cycloaddition [27] opens the possibility to automatize the functionalization of polymer with CAPs under physiological conditions [28]. These chemical reactions can also be used to crosslink natural [29] and synthetic polymers *in situ* [30] to afford hydrogels of different mechanical properties. CAPs terminated with orthogonal chemical moieties, such as amines and methyl sulfone can be immobilized onto polymers bearing thiol and carboxylic acid groups [31]. Alternatively, thiol-terminated CAPs can be immobilized onto maleimide-functionalized hydrogel-forming polymers such as PEG [32]. Since these reactions occur under physiological conditions and do not produce any by-products, no purification steps are required, and liquid handling robots can mix different CAPs, crosslinkers, and hydrogel precursors to form CAP-functionalized hydrogels. The automation of these fabrication steps allows for rapid and precise investigation of the concentration response of specific cell type to a given library of CAPs. Furthermore, an automation platform can rapidly investigate the synergistic effects of CAPs for a given cell type by mixing CAPs in different concentrations and combinations. From these cell culture platforms, more complex systems can be developed by integrating growth factors or substrates of various mechanical properties [33]. As such, materials that can be blended to afford substrates with different mechanical properties can be combined with coupling chemistries to automatize the screening of both mechanical environments and CAPs. As an example, molecular alloying of carboxylated agarose with native agarose in different ratios afforded hydrogels with different mechanical properties and could be implemented in an automated platform where a liquid handling robot blends the different hydrogel precursors [4]. Systems with tunable mechanical properties would promote the rapid manufacturing of a variety of cell

microenvironments and identify the role played by growth factors as a function of CAPs and mechanical properties. In turn, HTS of the main features of the ECM is expected to lead to accurate models of the natural ECM.

Implementing an automated platform to fabricate the cellular microenvironments and screening of the CAP impact is only one part of the challenge. Once manufactured, methods to determine the role of the CAPs need to be applied. While the cell viability on 2D substrate can provide a simple readout [34], more advanced characterization techniques are often required and could be translated from drug discovery platforms to the characterization of CAP-functionalized substrates. CAPs are involved in cell migration, spreading, and differentiation, thus readout techniques for these functions need to be applied to assess the CAPs (Box 1). Cell spreading requires microscopic techniques capable of rapidly and automatically measuring the cell elongation upon binding to CAPs. On 2D substrates, cell spreading can be determined by conventional microscopic techniques. However, on opaque or 3D substrates different instruments are required. Because opaque samples do not allow the use of conventional microscopy, upright fluorescent microscopes or scanning electron microscopes are required. By contrast, on 3D systems, hydrogels can be imaged using confocal laser microscopy, which can acquire successive focal layers through the samples. Comparatively, to acquire dynamic characteristics, such as cell migration, advanced living cell culture imaging capabilities are required. Such cell migration tracking techniques are now available on laboratory microscopes and even on smartphone-based systems. As an example, the movement of living spermatozoa on 2D substrate can be monitored and analyzed on a smartphone [35]. However, fully characterizing cell movement in 3D environments requires imaging systems able to track cell motility in space and over time, so-called **4D cell culture** [36]. These cutting-edge experiments result in a considerable amount of data that require automatized analysis of cell movements when comparing the behavior of a particular type of cells in different cell microenvironments. Yet, alternatives to the microscopic imaging can be implemented to characterize cells in their 3D cell microenvironment. Because the binding of CAPs to a cell receptor induces downstream **signaling pathway** activation, mRNA and protein analysis can be utilized to characterize the CAPs. One convenient way to rapidly monitor protein synthesis is to utilize transfected cells with a reporter gene that adds a fluorescent marker on the protein of interest [37]. While this technique is suitable when working with one specific cell type, it can be challenging to expand to the screening of large cell-type libraries in different cell microenvironments. Alternatively, the change in gene expression can be gathered by RT-PCR. This technique requires isolating and purifying the mRNA of the cells, and is widely used and established across laboratories. RT-PCR provides information on the gene profile and thus the cell fate in a process that can be fully automatized. Primarily developed for 2D cell cultures, this technique has been successfully translated into 3D cell culture platforms such as cell-embedded hydrogels [38]. Similar to the imaging of fixed samples, RT-PCR can only be applied for one time point per sample and thereby it considerably enlarges the number of samples needed when conducting experiments over several time points.

Despite the challenges to implement a completely automated platform for cell culture and its subsequent analysis, HTS of cell microenvironments has been reported in several examples. For instance, PEG hydrogels were used to identify 3D cell microenvironments to reproduce the stem cell niche as a function of material mechanical properties, cell concentration, soluble molecules, and macromolecules of the ECM [39]. Recently, photocured hydrogels were used to screen different CAPs as a function of cell density for ten cell types on a 3D microarray platform, demonstrating the feasibility of the HTS cell microenvironment approach [40].

The techniques applied in drug discovery HTS for experimental setup and characterization can be translated into the screening of CAPs and the cell microenvironment. Inspired by HTS platforms, the findings resulting from the screening of the cell microenvironment could feed back into the drug discovery process. This could offer tissue models for drug screening that reproduce healthy and diseased ECM environments of the same tissue by changing the composition of the synthetic ECM (Box 2).

Concluding Remarks and Future Outlook

Despite the many advances in material processing, coupling chemistries, and CAP discoveries, the screening of cell microenvironments remains on the periphery when engineering artificial tissue or medical devices (see Outstanding Questions). In addition, development in automated systems for liquid handling, microscopic imaging, and sample processing for mRNA extraction now provide many solutions to aid in the implementation of CAP screening. While automated systems can drastically reduce the time and manpower required for such experiments, the high costs of such systems might hinder widespread adoption of this approach. Nevertheless, the screening can be done manually on a smaller scale with promising results as demonstrated for MIN6 cells [16]. The prospect of developing a platform for the identification of CAPs beyond RGD has the potential to impact several fields in biomedical research (Figure 2, Key Figure). In the short term, results obtained from screening platforms can help to develop medical devices such as surfaces or scaffolds used in wound dressings or to develop implants with improved tissue integration [5]. Combining the results of CAP screening with recent advances in hydrogel design would have the potential to help answering fundamental questions. As an example, dynamic activation of peptides in a hydrogel matrix could further the understanding of these

Box 2. Disease-Driven ECM Changes

From the smoothness of the brain tissues to the toughness of bones, the mechanical properties of organs are considerably different. Such diverse environments are fabricated by cells by secreting the various ECM macromolecules in different proportions. These environments are tightly regulated between macromolecule deposition and degradation in a process called homeostasis. However, certain diseases or injuries can dysregulate this balance, and macromolecules in the diseased ECM can be over-represented (upregulation) or drastically reduced (downregulation). Therefore, engineering of tissue models that recapitulate the unbalanced ECM macromolecule composition would enable further investigation of the underlying mechanism of disease development such as cancer metastasis. Reproduction of the unbalanced cell-adhesion signals could allow for diverse diseases model to be developed. Therefore, to go beyond the integrin-binding RGD amino acid sequence, identification of CAPs that reproduce disease-specific ECM is required to develop such synthetic models (Table I). These models will have the potential to further improve the drug discovery process by allowing a more precise testing of drug candidate libraries.

Table I. Example of Composition Changes in the ECM during Injury or Diseases

Disease/injury	Tissue	Trend	Microenvironment	Refs
Cancer	Breast	↑[a]	Collagen I, II, III, IV, IX	[86]
Glial scar	Brain	↑	α1-Laminin-1 Collagen IV	[87]
Hepatic fibrosis	Liver	↑	Collagen I, III, and IV Laminin	[88]
		↓[b]	Elastin	
Ehlers–Danlos syndrome	Skin	↑	Collagen III	[89]
Chronic obstructive pulmonary disease	Lung	↑	Collagen	[90,91]
		↓	Elastin	

[a]↑ indicates upregulation.
[b]↓ indicates downregulation.

Key Figure

Potential Applications of Screening Cell-Adhesion Peptides

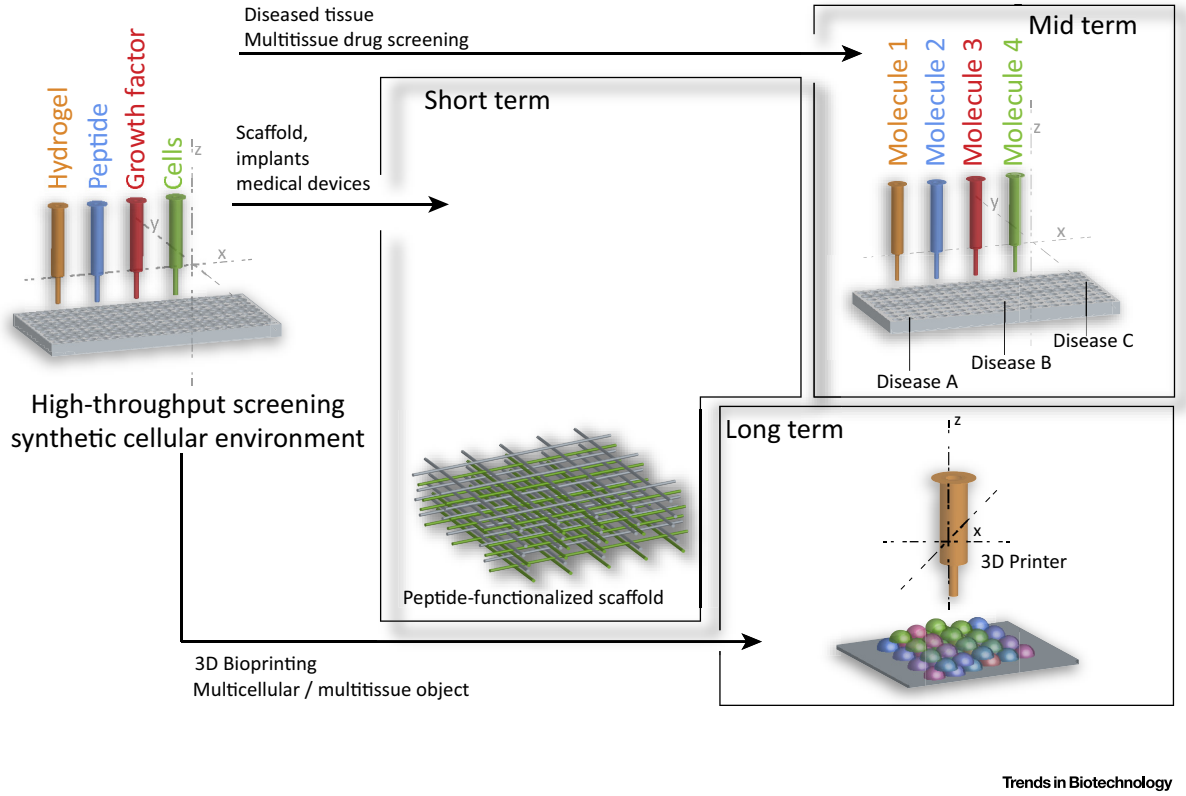

Figure 2. Some applications are in medical device design (short term), drug discovery (mid term), and bioprinting of tissues (long term) by biofabricating more complex and accurate cell microenvironments.

new CAPs [41], design of hydrogel matrices with specific CAPs could help to recapitulate different stages of organ formation [20], and specifically targeting a family of integrin receptors could allow for the precise direction of organ formation such as blood vessels [14]. Because 3D experiments are complex to design and characterize, the screening of 3D cell environments that could afford disease-specific models would require more time to feed back into the drug discovery process. Once established, these HTS platforms would have the potential to allow screening of drug candidate libraries on substrates mimicking different diseases or the comparison of diseases and healthy tissue on the same platform. Underlying these developments is the aim of reducing high costs and long development times associated with the drug discovery process and eventually reduce the use of animal models for preclinical testing. On a longer perspective, the field of **3D bioprinting** aiming at manufacturing complex functional tissue as an alternative to an allograft organ transplant could benefit from the proposed cell microenvironment screening for the development of **bioinks** [42].

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Supplemental Information

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Outstanding Questions

To what extent can cell fate and function be controlled by using CAPs other than the commonly used RGD, IKVAV, or YIGSR sequences?

Is high-throughput screening a viable approach to identify the optimal cell-adhesion peptides for a specific cell type?

Can cell-adhesion peptides help to reduce the use of growth factors to control cell differentiation and organization?

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