Published in final edited form as:

Matrix Biol. 2011 September ; 30(7-8): 363–368. doi:10.1016/j.matbio.2011.06.001.

Cell-Matrix Adhesions in 3D

Jill S. Harunaga and Kenneth M. Yamada

Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892

Abstract

Cells in a three-dimensional (3D) extracellular matrix environment often display different properties and behavior compared to cells cultured on a two-dimensional (2D) substrate. Recent studies characterizing the cell-matrix adhesions formed by cells within a 3D matrix have arrived at contradictory conclusions regarding the presence and composition of adhesions. Here we review this literature, and provide a comparative compilation of information found in published studies from the 3D cell-matrix adhesion field in order to identify shared and divergent conclusions and conceptually important areas that require further research. Although there is a general consensus that discrete cell-matrix adhesions exist in various 3D matrix environments, there are specific exceptions, particularly in cells undergoing amoeboid migration. There are also technical issues to consider when imaging adhesions in 3D matrix; for example, over-expression of a cytoskeletal cell adhesion component can potentially cloud the visualization of adhesions and even alter the mode of cell migration. Properties such as stiffness and local matrix topography may also affect the composition of cell-matrix adhesions. For example, even though cells contain integrin-based 3D adhesions, there can be substantial variability within these adhesions in the presence of force-dependent cytoskeletal components such as vinculin. These new findings and ideas provide promising new leads for understanding the regulation and function of cell-matrix adhesions in 3D matrix.

Keywords

cell adhesion; 3D extracellular matrix; cytoskeleton; integrin; vinculin; collagen

1. Introduction

The *in vivo* interactions between cells and their extracellular matrix microenvironments play many well-known roles in cell adhesion and migration, tissue remodeling and differentiation. Much of our mechanistic knowledge about these cell biological interactions is based on studies performed on rigid two-dimensional (2D) substrates. However, recent controversy about whether discrete cell adhesion structures actually exist at all in cells grown in a popular type of three-dimensional (3D) model system has raised interesting concerns regarding our understanding of the nature of cell-matrix adhesions, since their detection may depend on the methods used to study them. As we discuss below, even though the outcome of this debate seems reassuring in terms of much of the previous literature's

Corresponding author: Kenneth Yamada kenneth.yamada@nih.gov Tel.: 301-496-9124 Fax: 301-402-0897 30 Convent Drive Bldg 30, Room 426 Bethesda, MD 20892-4370 and Jill Harunaga jill.harunaga@nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

validity, the issues that it raises about the importance of the physical properties of the 3D extracellular matrix and the technical aspects of live-cell imaging have provided new ideas for future research.

Histological approaches and more-recent immunolocalization of specific molecules have provided fundamental knowledge about the structure and nature of cell interactions with the extracellular matrix. Cells in tissue culture adhere and interact with extracellular matrix molecules in a variety of ways, but they do so most strikingly through cell adhesion structures that include focal adhesions, fibrillar adhesions and other variants such as nascent adhesions, focal complexes and 3D-matrix adhesions, as well as the degradative structures termed podosomes and invadopodia (Dubash et al., 2009; Gardel et al., 2010; Geiger and Yamada, 2011; Parsons et al., 2010). These findings were established primarily using cells in regular tissue culture, using both immunohistochemical localization approaches and direct observation of fluorescent protein chimeras containing GFP (green fluorescent protein) expressed as a tag attached to various molecular components of cell-matrix adhesions. Studies using these regular flat (2D) cell culture substrates have provided innumerable important mechanistic insights, but they often fail to mimic morphological, signaling or other functional properties of cells and tissues in 3D (Friedl and Wolf, 2010; Grinnell and Petroll, 2010; Lee et al., 2008; Xu et al., 2009; Yamada and Cukierman, 2007).

Studies of cell adhesion structures in 3D are still in their infancy, but a recent search of the literature reveals nearly one dozen publications that have visualized discrete adhesion structures in cells within a 3D matrix. These 3D matrix adhesions contain molecules known to be present in 2D cell adhesions such as integrins, vinculin and paxillin, in both normal and cancerous cells. In order to integrate this body of literature, we have organized it into a comprehensive table with citations (Table 1). We also describe in this table the shape, size and localization patterns (or absence thereof) of cell adhesions in 3D matrix based on our visual inspection of the images published in these papers, because many such studies have not specifically described this important information.

Early studies reported that fibroblasts completely embedded within a thin cell-derived 3D matrix or in a collagen gel *in vitro* – as well as embryonic mesenchyme cells *in vivo* – have distinct cell-matrix adhesions containing both integrin and vinculin (Cukierman et al., 2001; Tamariz and Grinnell, 2002). More recent studies of primary human fibroblasts in different types of 3D matrix – fibrin and basement membrane extract/laminin, in addition to collagen and cell-derived matrix – have quantified the numbers and sizes of cell adhesions on cells embedded within 3D matrices (Hakkinen et al., 2011; Zhou et al., 2008). In addition, fibroblasts within soft gels comprised of basement membrane extract (Matrigel) lacked well-formed cell-matrix adhesions and were unable to migrate (Hakkinen et al., 2011). An important exception to these reports involves tumor cells or T lymphocytes that utilize an amoeboid form of migration to navigate within the 3D matrix that does not require the formation of integrin-based cell adhesions; instead, the cell adopts a bleb-based propulsive mode of migration. This process is distinct from a mesenchymal mode of migration that requires high levels of integrin-based cell-matrix adhesion and cytoskeletal contractility (Friedl et al., 1998; Sahai and Marshall, 2003; Wolf et al., 2003). In all of the other studies, however, cells displayed discrete cell adhesions in 3D matrices.

2. Visualizing adhesions in 3D

It can be argued, however, that the fixatives used on cells prior to immunofluorescence staining might induce aggregates that were interpreted to be cell adhesion structures in these studies, and that live-cell imaging is also essential. Petroll and Ma (2003) provided initial evidence suggesting that cell adhesions containing zyxin exist in living fibroblasts within a

3D collagen gel. Fraley, Wirtz and colleagues recently re-examined this question in depth using a variety of GFP chimeric reporters in cells within 3D collagen gels (Fraley et al., 2010). In striking contrast to much of the previous literature, they concluded that: “Live-cell microscopy shows that for cells fully embedded in a 3D matrix, focal adhesion proteins, including vinculin, paxillin, talin, α -actinin, zyxin, VASP, FAK and p130Cas, do not form aggregates but are diffusely distributed throughout the cytoplasm. Despite the absence of detectable focal adhesions, focal adhesion proteins still modulate cell motility...” They then described knockdown studies that show roles for these molecules in protrusion activity and matrix deformation even in the absence of distinct cell adhesions.

These findings were recently challenged by Kubow and Horwitz (2011) who, contrary to this report, were able to visualize and quantify cell-matrix adhesions in two different sarcoma cell lines in either rat tail collagen or pepsinized collagen gels, using a truncated CMV (cytomegalovirus) promoter. These authors raise an important technical issue, showing that conventional over-expression of fluorescent protein chimeras can result in an inability to visualize cell adhesions. This phenomenon is familiar to those who study cell adhesions in 2D, where non-physiological expression of adhesion plaque proteins can fill the cytoplasm and obscure visualization of discrete focal adhesions. In response to these findings, Fraley et al. (2011) state that they tried to avoid such issues, although they did so without using the “crippled” promoter used by Kubow and Horwitz to obtain low levels of chimeric protein expression. Because it is difficult to prove that a structure does not exist if others show that it exists (and quantify it), it is most likely safe to conclude that the live-cell imaging studies in this particular collagen gel 3D model system confirm the current literature showing the frequent presence of cell adhesions in 3D *in vitro* and *in vivo* using fixed samples.

Interestingly, an additional live-cell imaging study of cells in a different type of 3D matrix has been published recently that confirms the presence of 3D cell adhesions, but it identifies yet another potential problem resulting from the over-expression of labeled adhesion proteins. Analyzing a tumor cell line migrating in a 3D cell-derived matrix, Deakin and Turner (2011) show that GFP-talin identifies readily observable, discrete adhesions in this 3D environment. Intriguingly, however, over-expression of a different adhesion protein, GFP-paxillin, suppresses these 3D adhesions by switching the cells from mesenchymal migration to an adhesion-independent, amoeboid migration phenotype. This latter finding identifies yet another unexpected issue in live-cell imaging: exogenous over-expression of a focal adhesion protein can apparently switch the migratory phenotype of cells to a state in which 3D matrix adhesions no longer exist. Specifically, the authors suggest that a balance in the levels of the closely related proteins paxillin and Hic-5 can regulate mesenchymal versus amoeboid phenotypes and 3D matrix adhesions (Deakin and Turner, 2011).

In spite of these technical difficulties with the over-expression of tagged chimeric protein, Fraley et al. (2011) offer an interesting alternative explanation of the discrepancies, suggesting that the presence or absence of adhesions could be due to differences in the tension or elasticity within various regions of collagen gels. In support of this concept, Fraley et al. (2011) presented data suggesting that, at increasing distances from the glass substrate, cells in collagen gels showed a direct loss of detectable cell adhesions, with no adhesions detectable at distances greater than 200 μ m from the glass substrate. Kubow and Horwitz (2011) repeated this same experiment using very low-level expression of GFP-paxillin in the same type of collagen gel, and they found no such effect. Instead, they observed that the number of cell adhesions located as far away as 360 μ m from the substrate was remarkably similar to those found close to the substrate. Although factors such as differences in confocal microscope resolution and sensitivity could play a major role in these striking discrepancies, the simplest explanation may be that there were differences in the

expression plasmid used and that only very low expression of a fluorescent chimera was able to avoid obscuring localization in 3D adhesions.

3. Tension dependency of adhesions in 3D

Although conflicts in the literature remain, it does seem plausible that in a very soft 3D matrix formed by purified collagen, cells might be able to form integrin-based adhesions that lack certain mechanosensitive and/or cytoskeletal anchoring molecules, which could contribute to the difficulty of visualizing adhesions in 3D. A well-known principle established by studies of cell-matrix adhesions in 2D is that the formation of focal adhesions requires tension or force (Balaban et al., 2001; Pasapera et al., 2010; Riveline et al., 2001; Schiller et al., 2011). In fact, cells cultured on soft, pliable substrates display unstable focal adhesions (Pelham and Wang, 1997). In addition, vinculin, is known to accumulate near tiny integrin-based adhesions only after the application of force (Galbraith et al., 2002), indicating that vinculin accumulation is mechanosensitive. Cells have also been known to generate forces that match the rigidity of the matrix to which they adhere, up to a certain point (Solon et al., 2007). Therefore, in a very soft gel, cells would not generate much force and may not require as many mechanosensitive proteins in their adhesions.

There is emerging evidence that a similar force/tension dependency exists for certain adhesion components in 3D, though more in-depth studies are needed into the specific molecular composition of 3D matrix adhesions. Because of the extremely pliable nature of 3D collagen gels, it seems possible that cells in 3D are even more sensitive to their microenvironmental physical properties than cells on a 2D surface. For example, even though it was reported that there were well-formed 3D matrix adhesions on fibroblasts within fibrin gels and cell-derived matrix, a puzzling finding in the same study stated that vinculin was relatively difficult to detect in the cell-matrix adhesions of collagen gels, compared to the prominent detection of activated $\beta 1$ integrins in well-defined cell adhesions (Hakkinen et al., 2011). It will be important to evaluate whether the presence of integrins in adhesions is sufficient to provide cell-matrix interactions in the absence of adhesion plaque proteins such as vinculin and paxillin, which are usually readily detectable. In fact, even though their studies involved cells on top of a collagen gel rather than inside it, Petroll et al. (2003) found that cell adhesions containing zyxin exist in living cells on a 3D collagen gel, whose size and direction correlate with the extent of nearby matrix displacement. In other words, they are proportional to the apparent force being applied to the matrix, as predicted by other 2D studies on either rigid or flexible substrates (Pelham and Wang, 1997; Petroll et al., 2003). Tamariz and Grinnell (2002) directly compared the same cell type in collagen gels of two different densities (and thus stiffness), and they observed that cells in higher-density collagen gels have more protrusions with discrete cell-matrix adhesions containing vinculin. $\beta 1$ integrin, on the other hand, is present in both cases (Table 1). Using a different cell type with different concentrations of floating collagen, Provenzano and Keeley observed similar behavior, noting that both paxillin and vinculin were mostly cytoplasmic and the epithelial cells did not exhibit protrusions in lower concentrations of collagen, but that at a higher collagen concentration, the cells displayed protrusions containing cell-matrix adhesions (Provenzano et al., 2009).

We summarize in Figure 1 the evolving idea that even though cell-matrix adhesions containing $\beta 1$ integrin are formed by cells in various 3D matrix environments, vinculin may not be present in a particularly soft, compliant matrix such as a low-concentration collagen gel. In contrast, microenvironments with increased tension on adhesions due to higher density and stiffness of an extracellular matrix or stiff 2D substrates would promote the accumulation of vinculin in cell-matrix adhesions.

Cell-matrix interactions depend on bi-directional interactions between the stiffness of the matrix and intracellular contractility (Discher et al., 2005). Therefore, an alternative approach to understanding the role of cell-matrix tension in the formation and composition of cell adhesions in 3D is to induce cytoskeletal relaxation in cells using pharmacological inhibitors. Recent studies in 2D systems document changes in the components of cell adhesions after inhibition of myosin II-mediated contractility (Pasapera et al., 2010; Schiller et al., 2011; Wolfenson et al., 2011). Interestingly, even though many components of 2D cell-matrix adhesions such as vinculin, zyxin and paxillin are significantly diminished in integrin-based cell adhesions after inhibition of myosin II by blebbistatin, the molecular components that remained included integrins, talin, kindlin and filamin (Schiller et al., 2011). This result further supports the idea that $\beta 1$ integrins can be present while vinculin is absent or reduced in 3D adhesions of a soft, elastic 3D matrix in which there are likely to be lower contractile forces against the pliable substrate (Table 1). It is possible that in addition to vinculin, there are other adhesion components that differ depending on the elasticity of the 3D matrix. To test this interpretation, more studies need to be performed in 3D environments comparing inhibitors of contractility with variations in matrix composition and stiffness to determine the regulators of the molecular composition and function of 3D cell adhesions.

4. Influence of 3D matrix organization on adhesions

Another interesting variable in studies of cell adhesions in 3D involves the physical organization of the matrix itself. Although many studies have focused on relatively homogeneous collagen I gels, some matrices can be quite fibrillar *in vitro* and *in vivo* (Cukierman et al., 2001; Provenzano et al., 2008), and cells follow aligned fibers as they migrate *in vivo* (Nakatsuji and Johnson, 1984; Sidani et al., 2006). It may be easier to detect cell-matrix adhesions formed on well-defined fibrils *in vivo* than those on a less-organized fibrillar matrix *in vitro*. Consequently, studies of the nature of *in vivo* cell adhesions formed in direct response to the overall organization of the matrix substrate, i.e., fibrillar, planar or diffuse, while modulating the composition, local organization and stiffness of the matrix itself should provide further insight into the different types of cell-matrix adhesion. For example, the local topography of the matrix in terms of its physical shape and elasticity at micrometer or nanometer length scales may provide local control of the composition of a cell-matrix adhesion.

5. Perspectives

The recent publication of these partially contradictory ideas and conclusions has provided fresh ideas for future investigations, as was the case for many previous controversies in the literature. As reviewed above, recent principles, hypotheses, caveats and ideas that will need further evaluation include:

- The presence and composition of cell-matrix adhesions in 3D matrix micro environments can vary depending on the physical properties and composition of the matrix.
- Cells undergoing amoeboid migration in a 3D matrix can lack any integrin-based cell adhesions.
- Effective visualization of 3D matrix adhesions can be particularly sensitive to the level of over-expression of the fluorescent chimera.
- Even though cells form integrin-based adhesions in 3D, the stiffness of the matrix may determine whether certain components such as vinculin are present, or it may change the dynamics of adhesion components. Are these integrin-containing

contacts true cell adhesions, and/or do they have different physical characteristics? Which cytoskeletal and signaling molecules do they contain? Could these remaining components fulfill the basic requirements for adhesion, and are additional proteins recruited as matrix tension increases and the cell needs to generate more force in a 3D matrix?

- The expression level of a focal adhesion component can regulate the mode of migration and the nature of 3D matrix adhesions.
- The fibrillar or non-fibrillar organization of a 3D matrix may affect the type of cell adhesion formed. In addition, the local micro- or nano-topography of the matrix may also regulate cell adhesion composition.
- The bidirectional interaction of matrix stiffness and cytoskeletal contractility can determine the nature of cell adhesions not only on 2D substrates, but also within a 3D matrix. Is the signaling in each direction the same? Is one pathway more important than others in 3D?

Confirmation or revision of these tentative concepts in this rapidly developing field should provide exciting advances over the next few years in our understanding of the regulation and function of cell-matrix adhesions in 3D.

Acknowledgments

We thank Andrew Doyle, Emily Joo, and Ryan Petrie for helpful comments on the manuscript, Shelagh Johnson for editing, and the Intramural Research Program of the NIH, NIDCR for support.

References

- Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, Sabanay I, Mahalu D, Safran S, Bershadsky A, Addadi L, Geiger B. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat Cell Biol.* 2001; 3:466–472. [PubMed: 11331874]
- Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. *Science.* 2001; 294:1708–1712. [PubMed: 11721053]
- Deakin NO, Turner CE. Distinct roles for Paxillin and Hic-5 in regulating Breast Cancer cell morphology, invasion and metastasis. *Mol Biol Cell.* 2011; 22:327–341. [PubMed: 21148292]
- Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science.* 2005; 310:1139–1143. [PubMed: 16293750]
- Dubash AD, Menold MM, Samson T, Boulter E, Garcia-Mata R, Doughman R, Burridge K. Chapter 1. Focal adhesions: new angles on an old structure. *Int Rev Cell Mol Biol.* 2009; 277:1–65. [PubMed: 19766966]
- Fraley SI, Feng Y, Krishnamurthy R, Kim DH, Celedon A, Longmore GD, Wirtz D. A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nat Cell Biol.* 2010; 12:598–604. [PubMed: 20473295]
- Fraley SI, Feng Y, Wirtz D, Longmore GD. Reply: reducing background fluorescence reveals adhesions in 3D matrices. *Nature Cell Biology.* 2011; 13:5–7.
- Friedl P, Entschladen F, Conrad C, Niggemann B, Zanker KS. CD4+ T lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize beta1 integrin-independent strategies for polarization, interaction with collagen fibers and locomotion. *Eur J Immunol.* 1998; 28:2331–2343. [PubMed: 9710211]
- Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. *J Cell Biol.* 2010; 188:11–19. [PubMed: 19951899]
- Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. *J Cell Biol.* 2002; 159:695–705. [PubMed: 12446745]

- Gardel ML, Schneider IC, Aratyn-Schaus Y, Waterman CM. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu Rev Cell Dev Biol.* 2010; 26:315–333. [PubMed: 19575647]
- Geiger B, Yamada KM. Molecular architecture and function of matrix adhesions. *Cold Spring Harb Perspect Biol.* 2011 In press, doi:10.1101/cshperspecta007476.
- Grinnell F, Petroll WM. Cell motility and mechanics in three-dimensional collagen matrices. *Annu Rev Cell Dev Biol.* 2010; 26:335–361. [PubMed: 19575667]
- Hakkinen KM, Harunaga JS, Doyle AD, Yamada KM. Direct comparisons of the morphology, migration, cell adhesions, and actin cytoskeleton of fibroblasts in four different three-dimensional extracellular matrices. *Tissue Eng Part A.* 2011; 17:713–724. [PubMed: 20929283]
- Kubow KE, Horwitz AR. Reducing background fluorescence reveals adhesions in 3D matrices. *Nat Cell Biol.* 2011; 13:3–5. author reply 5–7. [PubMed: 21173800]
- Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: state of the art. *Tissue Eng Part B Rev.* 2008; 14:61–86. [PubMed: 18454635]
- Li S, Lao J, Chen BP, Li YS, Zhao Y, Chu J, Chen KD, Tsou TC, Peck K, Chien S. Genomic analysis of smooth muscle cells in 3-dimensional collagen matrix. *FASEB J.* 2003; 17:97–99. [PubMed: 12475912]
- Liu Y, Yanai R, Lu Y, Kimura K, Nishida T. Promotion by fibronectin of collagen gel contraction mediated by human corneal fibroblasts. *Exp Eye Res.* 2006; 83:1196–1204. [PubMed: 16914141]
- Martins GG, Kolega J. Endothelial cell protrusion and migration in three-dimensional collagen matrices. *Cell Motil Cytoskeleton.* 2006; 63:101–115. [PubMed: 16395720]
- Nakatsuji N, Johnson KE. Experimental manipulation of a contact guidance system in amphibian gastrulation by mechanical tension. *Nature.* 1984; 307:453–455. [PubMed: 6694737]
- Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol.* 2010; 11:633–643. [PubMed: 20729930]
- Pasapera AM, Schneider IC, Rericha E, Schlaepfer DD, Waterman CM. Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. *J Cell Biol.* 2010; 188:877–890. [PubMed: 20308429]
- Pelham RJ Jr. Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci U S A.* 1997; 94:13661–13665. [PubMed: 9391082]
- Petroll WM, Ma L. Direct, dynamic assessment of cell-matrix interactions inside fibrillar collagen lattices. *Cell Motil Cytoskeleton.* 2003; 55:254–264. [PubMed: 12845599]
- Petroll WM, Ma L, Jester JV. Direct correlation of collagen matrix deformation with focal adhesion dynamics in living corneal fibroblasts. *J Cell Sci.* 2003; 116:1481–1491. [PubMed: 12640033]
- Provenzano PP, Inman DR, Eliceiri KW, Keely PJ. Matrix density-induced mechanoregulation of breast cell phenotype, signaling and gene expression through a FAK-ERK linkage. *Oncogene.* 2009; 28:4326–4343. [PubMed: 19826415]
- Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ. Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J.* 2008; 95:5374–5384. [PubMed: 18775961]
- Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, Kam Z, Geiger B, Bershadsky AD. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J Cell Biol.* 2001; 153:1175–1186. [PubMed: 11402062]
- Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol.* 2003; 5:711–719. [PubMed: 12844144]
- Schiller HB, Friedel CC, Boulegue C, Fassler R. Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins. *EMBO Rep.* 2011; 12:259–266. [PubMed: 21311561]
- Sidani M, Wyckoff J, Xue C, Segall JE, Condeelis J. Probing the microenvironment of mammary tumors using multiphoton microscopy. *J Mammary Gland Biol Neoplasia.* 2006; 11:151–163. [PubMed: 17106644]

- Solon J, Levental I, Sengupta K, Georges PC, Janmey PA. Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophys J*. 2007; 93:4453–4461. [PubMed: 18045965]
- Tamariz E, Grinnell F. Modulation of fibroblast morphology and adhesion during collagen matrix remodeling. *Mol Biol Cell*. 2002; 13:3915–3929. [PubMed: 12429835]
- Vaughan MB, Howard EW, Tomasek JJ. Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. *Exp Cell Res*. 2000; 257:180–189. [PubMed: 10854066]
- Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Bocker EB, Friedl P. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J Cell Biol*. 2003; 160:267–277. [PubMed: 12527751]
- Wolfenson H, Bershadsky A, Henis YI, Geiger B. Actomyosin-generated tension controls the molecular kinetics of focal adhesions. *J Cell Sci*. 2011; 124:1425–1432. [PubMed: 21486952]
- Wozniak MA, Desai R, Solski PA, Der CJ, Keely PJ. ROCK-generated contractility regulates breast epithelial cell differentiation in response to the physical properties of a three-dimensional collagen matrix. *J Cell Biol*. 2003; 163:583–595. [PubMed: 14610060]
- Xu R, Boudreau A, Bissell MJ. Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. *Cancer Metastasis Rev*. 2009; 28:167–176. [PubMed: 19160017]
- Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. *Cell*. 2007; 130:601–610. [PubMed: 17719539]
- Zhou X, Rowe RG, Hiraoka N, George JP, Wirtz D, Mosher DF, Virtanen I, Chernousov MA, Weiss SJ. Fibronectin fibrillogenesis regulates three-dimensional neovessel formation. *Genes Dev*. 2008; 22:1231–1243. [PubMed: 18451110]

- Adhesion containing integrins
- Adhesion containing both integrins and vinculin

Figure 1.

Working model of 3D cell-matrix adhesions in different environments. On a rigid 2D surface, cells adhere firmly to the substrate using adhesions that contain both integrins and vinculin. In a very soft low-density collagen gel, cells can still form integrin-containing adhesions, but the adhesions are smaller and reduced in number, and fewer are vinculin-positive. In contrast, in a stiffer high-density collagen gel, the cells form more adhesions that are more vinculin-positive. Moreover, in a stiff 3D environment such as cell-derived matrix or a fibrin gel, all of the 3D cell-matrix adhesions contain both vinculin and integrins.

Table 1

Characterization of 3D cell-to-extracellular matrix adhesions based on the current literature. The authors have compiled this table based on direct comparisons of published images of different cell types in various 3D matrix environments. The table describes the shape, size, and localization patterns of different cell adhesion components. This comprehensive table allows for direct comparisons of similarities and discrepancies between data in the literature to order to identify areas requiring further investigation.

Publication	Cell type	Matrix	Protein	Adhesion shape and location
Friedl et al., 1998	CD4 ⁺ T lymphocytes (spontaneously migrating)	Collagen (1.67 mg/ml rat tail)	Integrin $\beta 1$ (mAb: K20)	Dim cortical localization, no adhesions visible
Vaughan et al., 2000	Myofibroblasts	Collagen (0.65 mg/ml rat tail collagen)	Vinculin	Dim elongated adhesions along cortex and under cell body; increased in length and number after TGF- β treatment
Cukierman et al., 2001	Human foreskin fibroblasts = HFF	3T3-cell derived matrix = 3T3-CDM	Integrin $\alpha 5$	Large elongated adhesions that co-localize with fibronectin fibers
	HFF	3T3-CDM	Paxillin	Large elongated adhesions that co-localize with fibronectin fibers
Tamariz and Grinnell, 2002	HFF (low density)	Collagen (1.5 mg/ml pepsin treated)	Vinculin	Diffuse staining, no visible adhesions with or without LPA or PDGF
	HFF (high density)	Collagen (1.5 mg/ml pepsin treated)	Vinculin	Large puncta located both in protrusions and the cell body with LPA or PDGF treatment
	HFF (low density)	Collagen (1.5 mg/ml pepsin treated)	Integrin $\beta 1$ (mAb: 9EG7 [*])	Very small puncta, concentrated on the main cell body, with small amount located in protrusions with or without LPA or PDGF
	HFF (high density)	Collagen (1.5 mg/ml pepsin treated)	Integrin $\beta 1$ (mAb: 9EG7)	Medium-sized puncta, some elongated, mostly on the main cell body, less in protrusions with or without LPA or PDGF
Wolf et al., 2003	HT1080 fibrosarcoma (mesenchymal)	Collagen (1.67 mg/ml pepsin treated)	Integrin $\beta 1$ (mAb: K20)	Small puncta at leading edge and around the cell cortex; the adhesions coincide with collagen fibers and co-cluster with MT-1
	HT1080 (ameboid)	Collagen (1.67 mg/ml pepsin treated)	Integrin $\beta 1$ (mAb: K20)	"Linear surface distribution" around cortex of cell, no punctate adhesions visible
Li et al., 2003	Vascular smooth muscle cells = SMC	Collagen (1 mg/ml rat tail)	Vinculin	Small puncta with occasional elongated adhesions localized mostly at the front of cells, smaller and fewer compared to adhesions on 2D
Petroll et al., 2003	Rabbit corneal fibroblasts = TRK-36 (live)	Collagen (2.48 mg/ml pepsin treated)	Zyxin	Elongated adhesions "most concentrated along the pseudopodia at the leading edge of cells" found along collagen fibers and microfilament bundles as well as scattered in cell body
Wozniak et al., 2003	T47D breast carcinoma cells	Collagen (1.3 mg/ml rat tail)	Vinculin	Cells in attached collagen gels form some protrusions that contain small punctate adhesions
	T47D breast carcinoma cells	Floating collagen (1.3 mg/ml rat tail)	Vinculin	Cells in floating collagen gels form tubules and do not have any protrusions; vinculin staining is cortical and not in punctate adhesions

Publication	Cell type	Matrix	Protein	Adhesion shape and location
Martins and Kolega, 2006	Bovine aortic endothelial cells	Collagen (3 mg/ml pepsin treated)	Vinculin	Small puncta in cell protrusions
	Bovine aortic endothelial cells	Collagen (3 mg/ml pepsin treated)	Paxillin	Small puncta in cell protrusions
Liu et al., 2006	Primary human corneal fibroblasts	Floating collagen (1.8 mg/ml pepsin treated)	Paxillin	Punctate adhesions in the front and rear protrusions of the cell with some elongated adhesions in the cell body; with the addition of FN cells become more spread and adhesions increase in number and size
Zhou et al., 2008	Endothelial cells from human umbilical cord	Fibrin	Integrin $\beta 1$ (activated)	Small and elongated “stitch-like” adhesions at edges of cell, larger and elongated under cell body
	Endothelial cells	Fibrin	Vinculin (transfected)	Consistently small in size, slightly elongated at periphery of cells
Provenzano et al., 2009	Normal murine mammary epithelial cells = NMuMG	Floating collagen gels (3 mg/ml rat tail)	Paxillin	No visible puncta in low-density collagen
	NMuMG	Floating collagen gels (4 mg/ml rat tail)	Paxillin	More prominent cytoplasmic localization compared to vinculin; small punctate adhesions at the tips of protrusions in this high-density collagen.
	NMuMG (live and fixed)	Floating collagen gels (3 mg/ml rat tail)	Vinculin	Cells in low density collagen are more rounded and tube-shaped with no protrusions, they contain less overall vinculin compared to high density; there are some small puncta in the cell body
	NMuMG (live and fixed)	Floating collagen gels (4 mg/ml rat tail)	Vinculin	Cells in high-density collagen have more protrusions, which contain a few elongated adhesions
Fraley et al., 2010	HT1080 (live)	Collagen (2 mg/ml rat tail)	Vinculin	Diffuse and cytoplasmic with no distinct aggregates of protein
	HT1080 (live)	Collagen (2 mg/ml rat tail)	Zyxin	Diffuse and cytoplasmic with no distinct aggregates of protein
Hakkinen et al., 2011	HFF	3T3-CDM	Integrin $\beta 1$ (mAb: 9EG7)	Small and punctate at front, elongated under cell body
	HFF	3T3-CDM	Vinculin	Fewer than when stained for $\beta 1$, mostly small puncta, with a few elongated adhesions
	HFF	Collagen (2.5 mg/ml pepsin treated)	Integrin $\beta 1$ (mAb: 9EG7)	Very prominent adhesions, both small punctate and elongated adhesions are present
	HFF	Collagen (2.5 mg/ml pepsin treated)	Vinculin	Adhesions rarely under cell body, mostly thin elongated adhesions located around the periphery of the cell
	HFF	Fibrin	Integrin $\beta 1$ (mAb: 9EG7)	Elongated adhesions located all around the cell
	HFF	Fibrin	Vinculin	Small puncta along the cell periphery
	HFF	BME	Integrin $\beta 1$ (mAb: 9EG7)	Small puncta under the cell body

Publication	Cell type	Matrix	Protein	Adhesion shape and location
	HFF	BME	Vinculin	No adhesion staining visible
Fraley et al., 2011	HT1080 (live)	Collagen (2 mg/ml rat tail)	Paxillin	No adhesion staining visible greater than 200 μ m away from the bottom of the dish
	HT1080 (live)	Collagen (2 mg/ml rat tail)	Zyxin	No adhesion staining visible greater than 200 μ m away from the bottom of the dish
Kubow and Horwitz, 2011	HT-1080 (live)	Collagen (2 mg/ml pepsin treated or rat tail)	Paxillin	Cell are multipolar with small puncta in each of the protrusions
	U2OS osteosarcoma (live)	Collagen (2 mg/ml pepsin treated)	Paxillin	Small elongated adhesions that coincide with collagen fibers at the leading edge and around the cell cortex
	U2OS osteosarcoma (live)	Collagen (2 mg/ml pepsin treated)	Vinculin	Small, elongated adhesions when coinciding with collagen fibers, only visible at leading/trailing edges
Deakin and Turner, 2011	MDA-MB-231 breast carcinoma cells	HFF-CDM	Paxillin	Smaller adhesions towards the front of the cell and larger, elongated adhesions towards the rear; adhesions coincide with fibronectin fibers
	MDA-MB-231	HFF-CDM	Vinculin	Many elongated adhesions predominantly at the front of the cell, very few at the rear of the cell
	MDA-MB-231 (live)	HFF-CDM	Talin	Many small elongated adhesions concentrated at the front and around the cell cortex, some longer ones are located under cell body, fewer at cell rear

* Notes on integrin monoclonal antibodies (mAb): 9EG7 detects activated and/or ligand-occupied β 1, while K20 is a non-perturbing anti- β 1 antibody