

You Talking to Me? Cadherin and Integrin Crosstalk in Biomaterial Design

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While much work has been done in the design of biomaterials to control integrin-mediated adhesion, less emphasis has been put on functionalization of materials with cadherin ligands. Yet, cell–cell contacts in combination with cell–matrix interactions are key in driving embryonic development, collective cell migration, epithelial to mesenchymal transition, and cancer metastatic processes, among others. This review focuses on the incorporation of both cadherin and integrin ligands in biomaterial design, to promote what is called the “adhesive crosstalk.” First, the structure and function of cadherins and their role in eliciting mechanotransductive processes, by themselves or in combination with integrin mechanosensing, are introduced. Then, biomaterials that mimic cell–cell interactions, and recent applications to get insights in fundamental biology and tissue engineering, are critically discussed.

integrins are mechanotransducers: the properties of the ECM can also be sensed by the cells and converted into biochemical signals. The combination of these cadherin- and integrin-mediated processes allows cells to create a landscape of their surroundings.

Both types of adhesions activate a myriad of signaling pathways that control the organization of the cellular cytoskeleton, signal transduction, and activation of transcriptional events, which ultimately drive proliferation, differentiation, and migration, among other cellular processes.^[5] Due to the complex interplay between cell–cell and cell–ECM adhesions, isolating and studying the signaling pathways in which they are involved can prove to be a challenging task. Hence, a range of biomaterials,


with controlled chemical, physical, and geometrical characteristics, have emerged as platforms to study cadherin signaling and the cadherin–integrin crosstalk. Crucially, while these studies have contributed to elucidate critical signaling pathways and signal integration between cadherin- and integrin-mediated adhesion, they have also paved the way to establishing a new paradigm in biomaterials’ design, where these interactions are harnessed to control cell behavior, from the control of collective cell migration, to paracrine secretion and cell differentiation for regenerative medicine applications.

In this review, we first describe the signaling pathways involved in cell–cell adhesion and in the integrin–cadherin adhesive crosstalk, as well as their implications in cell behavior and fate. Then, biomaterials that have been designed to study cadherin or adhesive crosstalk signaling are presented, and the various approaches that have been proposed to harness the (regenerative) potential of these adhesive interactions are discussed.

1. Introduction

Cell–cell adhesions within tissues are largely mediated by cadherins, adhesion proteins that play a crucial role in development, wound healing, and tissue homeostasis.^[1,2] This superfamily of calcium-dependent transmembrane proteins establish homotypic interactions with adjacent cells in a zipper-like manner,^[3] forming “adherens junctions” that link these adhesions to the cell cytoskeleton. Via this linkage, cadherins are able to sense forces and activate mechanotransductive signaling cascades.^[4] While cadherins are responsible for cell–cell interactions, integrins are the main transmembrane receptors in charge of the interaction between cells and their extracellular matrix (ECM). In order to establish these connections, integrins cluster and recruit other intracellular proteins, forming focal adhesions. Like cadherins,

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2. Cadherin Structure and Molecular Interactions

Cadherins are a superfamily of transmembrane receptors which exist in a wide range of species, from unicellular animals^[6] to mammals, where they are involved in important processes such as morphogenesis,^[7] cell signaling,^[8] physical homeostasis,^[9] and mechanotransduction.^[10] They can create different kinds of cell–cell adhesions, such as desmosomes and adherens junctions (AJs). The former are among the strongest cell–cell adhesion types: they can be found in highly mechanically stressed tissues,^[11] where they are arranged on the lateral sides of plasma

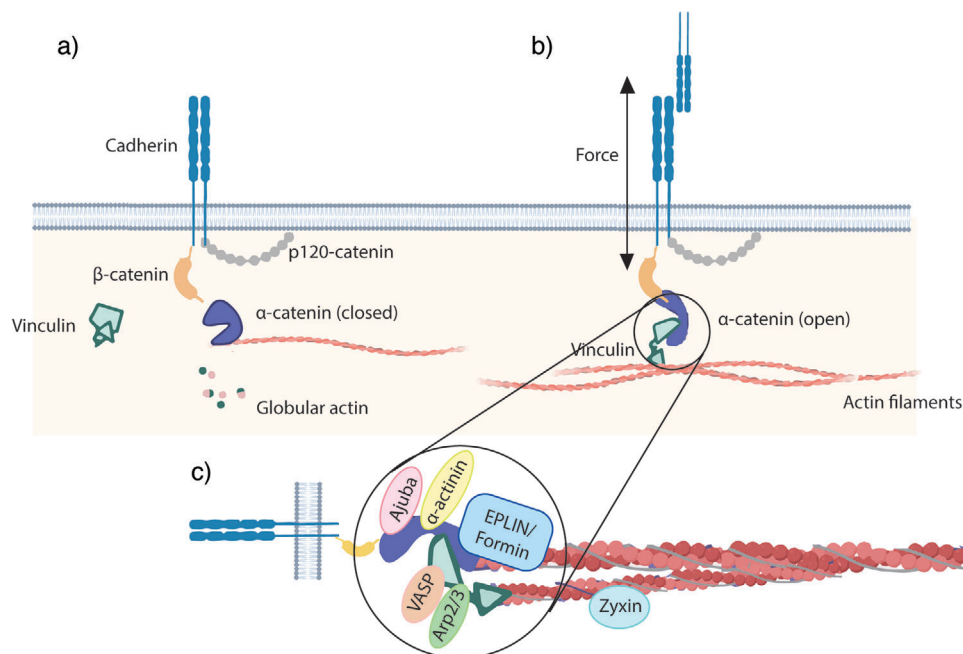


Figure 1. a) Cadherin-mediated adhesion without tension. α -catenin remains closed with the vinculin-adhesion site covered. It can still bind actin. Vinculin remains in its autoinhibited form. b) When force is applied, α -catenin opens showing the vinculin-binding site. Vinculin changes its conformation attaching to α -catenin and being able to bind F-actin. c) Several proteins are recruited, promoting actin polymerization, myosin contractility, and subsequent reinforcement of the junction.

membranes and linked to the intermediate filament network. The latter are the main cell–cell structure that responds to mechanical stimuli,^[9] being involved in the initiation and stabilization of the actin cytoskeleton, in intracellular signaling and transcriptional regulation. They are linked to the cytoskeleton via the cadherin– β -catenin– α -catenin complex. Other cell–cell adhesions, such as the tight junctions encountered in epithelial cells, are instead noncadherin-based.^[12] In these multiprotein complexes, which prevent the mixing of membrane proteins between the apical and basolateral membrane and control the passage of ions between cells, occludins, and claudins are in charge of the cell–cell adhesion.^[13]

The cadherin superfamily shares a common structural component named the extracellular cadherin (EC) domain, formed by ≈ 110 amino acid residues. Cadherins are classified according to the number and arrangements of these domains. The most studied cadherin families include the classical cadherins, which create AJs and are expressed in a tissue specific manner: E-cadherin, expressed in epithelia, N-cadherin, in nonepithelial cells, Vascular-Endothelial (VE)-cadherin, in vascular tissues, and P-cadherin, in myoepithelial cells. Such classical cadherins comprise a cytoplasmic domain, a transmembrane domain, and an extracellular domain (ectodomain) containing five EC domains. They establish homophilic cell–cell adhesion stabilized by calcium,^[14] which serves to rigidify the cadherin extracellular domains promoting a rod-like conformation that creates junctional interactions. More precisely, three calcium ions can bind with different affinities to each one of the pockets between cadherin extracellular domains, but only two of them appear to play a role in this rigidification.^[15,16] When calcium is depleted, a partial loss of interaction between N-cadherin molecules

has been observed.^[17] Simulations also confirm that in the absence of this ion the interdomain junctions become flexible, which avoids *cis*-dimer formation.^[16] The most distal domain, EC1, contains the adhesive sequence in charge of making contact with other cadherins, such as HAVDI for N-cadherin or HAV for E-cadherin.^[18,19] The extracellular cadherin domains are sufficient to create initial contacts even without the intracellular domain.^[20,21] Nevertheless, the cytoplasmic domain is crucial for the strengthening of the adhesion, thanks to the actin rearrangements near the junction^[22] via interaction with catenins.^[4]

In 1989, Ozawa et al. discovered that the cytoplasmic region of E-cadherin was in contact with β -catenin and p120-catenin.^[23] Subsequent characterization demonstrated that the high level of sequence identity between classical cadherins was responsible for the interaction of other cadherins (N- and P-cadherins) with β -catenin, which binds to α -catenin.^[24] β -catenin, a highly conserved protein, present in insects and vertebrates, is also a transcriptional coactivator in the Wnt signaling pathway, crucial during embryogenesis and tissue renewal.^[25] p120-catenin instead regulates cadherin levels by controlling cadherin turnover^[26] and also controls small guanosine triphosphate (GTP)ases that regulate the actin cytoskeleton^[27] (Figure 1a,b).

α -catenin has multiple binding partners, being β -catenin and F-actin some of them,^[28] and as a consequence, linking the cadherin/ β -catenin complex to the actin cytoskeleton.^[29] It binds to actin filaments and vinculin in a tension-dependent manner.^[30] To be able to bind both β -catenin and F-actin, this protein has to experience force-dependent conformational changes. These changes explain the transition between its weak and strong bound states with F-actin.^[31] Without tension, α -catenin adopts an autoinhibited form, in which its vinculin-binding site is

masked (Figure 1a). On the other hand, when tension is exerted, force-induced conformational changes uncover the vinculin-binding site. This strengthens the interaction with vinculin,^[32] which not only makes contact with actin but also stabilizes the α -catenin open conformation (Figure 1b,c). Once tension stops, after a sustained force-dependent activation of α -catenin, it is not clear if the protein refolds or if it undergoes other cellular processes such as proteolysis.^[33]

Vinculin is a widely studied mechanosensor not only at AJs, but also at integrin-mediated adhesions. Its three domains confer vinculin the capacity to bind α -catenin, β -catenin, talin, as well as some regulators of the actin cytoskeleton such as vasodilator-stimulated phosphoprotein (VASP or Arp2/3 (in the case of the head domain)). The tail can also directly bind F-actin or paxillin and its own head.^[34] Without tension, vinculin is located in the cytoplasm in an autoinhibited form, but when there is an increase in force, it is recruited by the tension-activated α -catenin,^[35–37] reinforcing and stabilizing the AJs. Once vinculin is recruited and activated, it can also bind β -catenin, allowing the recruitment of additional vinculin molecules that reinforce the adhesion.^[38]

3. Cadherin-Mediated Mechanotransduction

The adhesive connections formed by cadherins are intrinsically mechanosensitive due to their ability to resist to forces generated inside the cell (due to the actin cytoskeleton machinery) or outside it (e.g., due to fluid shear stress or tissue deformation). Although cadherins by themselves are able to trigger biochemical signaling events such as the control of organ size or protection from apoptosis,^[39] they can also link cytoskeletons of neighboring cells, creating connections that allow mechanical communication among cells. AJs are the principal cell–cell structure that reacts to forces and are crucial during embryogenesis, tissue morphogenesis, and for the integrity of adult tissues. AJs can withstand forces of ≈ 50 – 300 pN *in vivo*^[40] and ≈ 50 – 100 nN *in vitro*;^[41] the differences in the magnitude can be due to the height of the junctions and density of the complexes. As mentioned in the previous section, some of the proteins constituting the AJs, such as vinculin or α -catenin, are mechanosensitive: they activate and/or stabilize under tension. Indeed, α -catenin has been recognized to be the central molecule in cadherin-mediated mechanotransduction.^[10] α -catenin unfolds under tensions of ≈ 5 pN^[32] and each cadherin–catenin complex is subjected to a tension of ≈ 2 – 5 pN under resting conditions and ≈ 50 pN in stressed conditions.^[42] Importantly, the strength of cadherin-based adhesions depends on the local coupling of cadherin clusters to actin filaments.^[43]

Other proteins besides vinculin that bind to α -catenin and also play a role in mechanotransduction are formins (mDia1, mDia2, and formin-1), which are recruited by α -catenin through the activation of RhoA at AJs. Formin recruitment produces the stabilization and organization of the actin cytoskeleton linked to the junction, giving rise to a reinforcement due to a positive feedback mechanism for AJ remodeling.^[44] Another protein that binds to α -catenin and vinculin is α -actinin, which stabilizes AJs creating actomyosin-derived tension, thanks to its interaction with myosin II and vinculin recruitment at AJs.^[45] The ajuba members (LIM domain-containing protein 1 (LIMD1) and WT1 Interacting Protein (WTIP)) are another family of proteins recruited

by α -catenin, known to regulate the Hippo pathway, and therefore the Yes-associated protein (YAP)/WW-domain-containing transcription regulator 1 (WWTR1 or TAZ) mechanical rheostat, by inhibiting large tumor suppressor kinase 1 (LATS) and allowing YAP translocation to the nucleus.^[46] Finally, Epithelial proteins lost in neoplasm (EPLINs) work as additional linkers between α -catenin and the cytoskeleton although they are not needed for direct force transmission (Figure 1c).^[47]

Mechanosensing via cadherins goes beyond the proteins that interact with α -catenin. There are actomyosin remodeling proteins that are recruited and/or activated at AJs in a α -catenin-independent manner. Some examples are myosin proteins, which activate RhoA at the junctions (myosin VI),^[48] contribute to the transmission of forces in nascent AJs (myosin IIB), or promote tensile forces through the formation of stress fibers (myosin IIA).^[49,50] Others are actin remodeling proteins, such as VASP, zyxin, and testin (TES), which are recruited to AJs in a tension-dependent manner.^[51] Similar to mechanotransductive integrin-based adhesions, while AJs are formed by the core structural and mechanosensitive components described above, the number of signaling molecules implicated in cadherin-based mechanotransduction is much greater. Indeed, when actomyosin remodeling and cytoskeletal dynamics are involved, it is crucial to consider the role of Rho (RhoA, Rac, and Cdc42) and Rap family GTPases, which are critical for cadherin-mediated adhesion^[52] and collective migration.^[27] More concretely, RhoA, Rac1, and Cdc42, regulated by p120-catenin, have been demonstrated to be the key regulators in the assembly, disassembly, remodeling, and contractility of the actomyosin cytoskeleton. Rac1 and Cdc42 are in charge of nucleation, polymerization, and stabilization of actin filaments,^[22] while RhoA is involved in actin regulation as well as myosin contractility through Rho-associated protein kinase (ROCK).^[53] In Chinese hamster ovary (CHO) cells, C-cadherin adhesion and engagement resulted in increased Rac1 activity and RhoA inhibition.^[54] This increase in the activity of Rac1 was also observed in epithelial cells, where overexpression of Rac1 or Cdc42 increased E-cadherin localization, whereas dominant negative mutants of both Rac1 and Cdc42 inhibited these events.^[55,56]

All of these AJ reinforcements, together with actomyosin remodeling, provide cells with the necessary information to regulate embryo morphogenesis,^[57] barrier maintenance,^[48] proliferative control in adult tissue, or collective migration.^[58] Also, mechanical forces that are sensed via the cell–cell junctions provide a mode of cell–cell communication that complements better understood modes of communication such as gap junctions or secreted factors.

4. YAP/TAZ

As suggested in the previous sections, the conversion of cadherin-mediated mechanical stimuli into biochemical signals plays a crucial role in important cell processes such as tissue remodeling,^[59] collective migration,^[60] or cell fate,^[61] among others. One of the main components of the mechanotransductive pathways is YAP/TAZ, which is known for its ability to act as a mechanical rheostat via its translocation to the nucleus in response to some mechanical inputs such as cell geometry,^[62] stretching, ECM stiffness,^[63] adhesion,^[64] and shear stress.^[65]

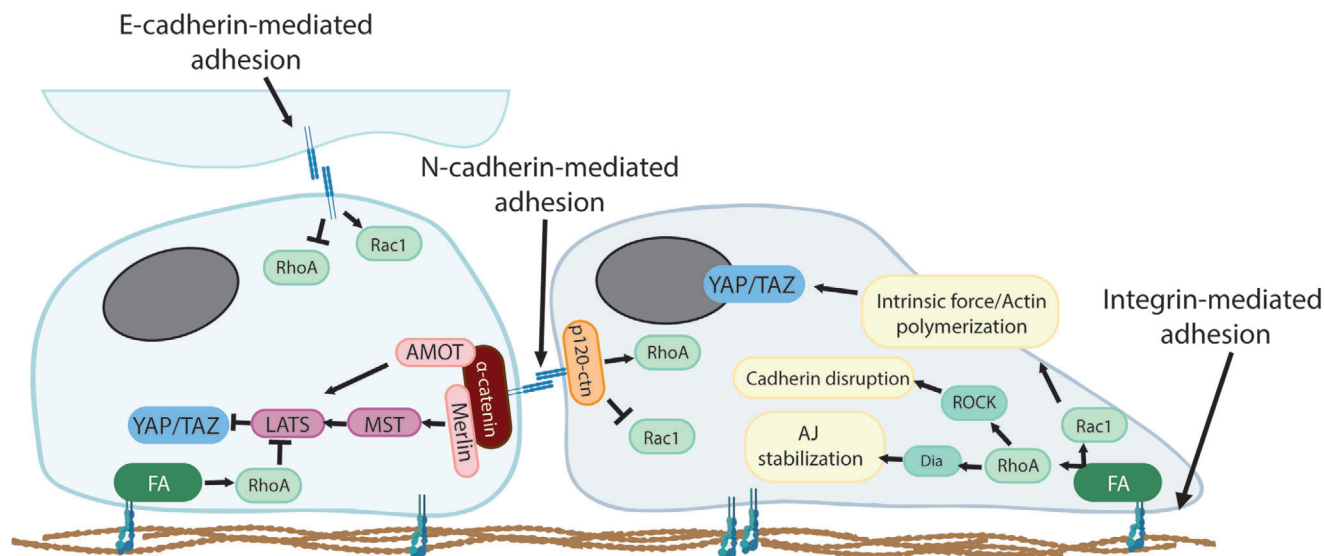


Figure 2. E-cadherin adhesion showing inhibition of RhoA and activation of Rac1 activity. N-cadherin-mediated adhesion in the cell of the left also shows the inhibition of YAP translocation to the nucleus due to the activation of MST via Merlin, activation of LATS, and subsequent phosphorylation of YAP/TAZ. LATS activity is also regulated by AMOT which can bind AJs. FA formation also activates GTPases which inhibit LATS, increasing YAP translocation to the nucleus. In the cell on the right, inhibition of Rac1 and activation of RhoA by p120-catenin at the cell–cell junction in N-cadherin-mediated adhesions are shown. On the other hand, integrin-mediated adhesions activate Rac1 at the free end of the cell, provoking actin polymerization and increase in intrinsic force. The activation of RhoA via integrins can also activate ROCK, increasing myosin activity and producing cadherin disruption. Conversely, activation of Dia via RhoA stabilizes AJs.

YAP/TAZ is also the central effector of the Hippo signaling pathway, which negatively regulates the activity of YAP/TAZ by promoting its cytoplasmic retention by 14-3-3 proteins and subsequent degradation in a mechanical-independent way. YAP recruiting to the cytoplasm is provoked via the LATS, which is activated by mammalian Ste20-like kinases 1/2 (MST).^[66] Although the signal transduction cascade of the core player kinases of the Hippo pathway has been intensively studied, the upstream components are not well understood.^[67] In 2011, Kim et al. demonstrated that the homophilic interaction among E-cadherins was one of the direct stimulators of the Hippo pathway via a catenin-dependent process, which activates Merlin (also known as neurofibromin 2), MST, and finally LATS, leading to phosphorylated and inactivated YAP (Figure 2, left cell).^[68]

The Hippo pathway is also influenced by architectural and mechanical cues caused by F-actin and myosin II interactions.^[69,70] Among the essential mediators connecting mechanical stimuli and Hippo–YAP regulation are Rho GTPases, which are known to be some of the main effectors in actin remodeling.^[66] It has been demonstrated that RhoA, Cdc42, and Rac1 all enhance YAP activity, although to a lesser extent in the case of Cdc42 and Rac1.^[63,71] Attachment to other cells via AJs also provokes tension in the actomyosin cytoskeleton, promoting E-cadherin-dependent YAP activation and subsequent cell proliferation.^[72]

On the other hand, contraction of the actin belts located in the cytoplasmic part of the AJs suppresses nuclear translocation of YAP by the liberation of Merlin from AJs, which exports YAP from the nuclei.^[68,73] It is thought that Merlin provides a regulated linkage between transmembrane proteins and the actin cytoskeleton,^[74] whose disruption increases Merlin interaction with LATS, producing YAP phosphorylation and inactivation.^[75] Another actin-binding protein which regulates YAP activity by

both phosphorylating it or sequestering it to the junctions is angiominin (AMOT), which also binds to and interacts with AJ components.^[76] The last actin-dependent kinase involved in the Hippo pathway is LATS1/2. Some recent studies have shown that LATS does not play a critical role in actin-dependent mechanotransduction because its inhibition does not rescue YAP inhibition by a soft environment.^[77] Nevertheless, it has been proposed that LATS activity is sensitive to mechanical cues due to its activation by protein kinase A (PKA) (downstream of the actin cytoskeleton).^[78]

5. Crosstalk with Extracellular Matrix Mechanosensing

ECM mechanosensing is mainly conducted by integrins, which are transmembrane receptors that interact with the ECM via adhesive sequences such as the Arg-Gly-Asp (RGD) motif.^[79] Integrins are part of the cell adhesion machinery, which consists of specialized subcellular contact sites where transmembrane receptors are in contact both with the ECM and the cytoskeleton. This machinery creates macromolecular assemblies called focal adhesions (FAs), through which mechanical and regulatory signals are transmitted to the cells.^[80] For the cells to be able to convert nascent adhesions into FAs, the recruitment of other components is required, as this will reinforce integrin–cytoskeleton bonds.^[81–83]

Within FAs, tension is sensed via a change in the conformation of talin, a protein that links the cytoplasmic tail of integrins to F-actin. When mechanical force is applied to talin, this protein changes its conformation exposing a vinculin binding domain, in such a way that vinculin is translocated to the focal adhesion producing FA reinforcement.^[84] It is also known that when talin

is activated, F-actin stabilizers and elongators are recruited provoking its polymerization and, as a consequence, cell adhesion to the ECM and spreading.^[85] The molecules described above belong to the “molecular clutch,” a concept that was proposed for the first time in 1988 by Mitchison and Kirschner to explain how the actin retrograde flow exerts tension on a substrate.^[86] For a deeper understanding of the molecular clutch model and of integrin-mediated mechanotransduction, the reader can refer to recent reviews.^[87–89] Mechanotransduction via integrins also recruits paxilin and focal adhesion kinases (FAKs); these work as a scaffold for numerous other proteins that regulate Rho GTPases, promoting stress fiber formation and myosin II activity through the activation of ROCK, and binding Rho family regulatory proteins.^[90]

Some of the proteins observed at cell–ECM adhesions are also present in cell–cell adhesions, creating a converging signaling network called “adhesive crosstalk,” whose regulation is not fully understood yet. This term is used to highlight crosstalks between two types of adhesions, based on integrins and cadherins in this case, which can interact at different levels. Both integrins and cadherins are transmembrane receptors, which are spatiotemporally controlled. Spatially because they both need activation through the presence of integrin-adhesion domains or other cadherins, which is determined by the presence of ECM or other cells. The temporal regulation comes with the dynamic processes that involve cadherin and integrin activation such as changes in protein conformation, cluster formation, or activation of downstream effectors.^[91–93] These receptors also share signaling molecules, scaffolding and cytoskeletal elements, and the ability to influence cell growth, survival, and transcriptional activity.^[5]

5.1. Signal Integration

Vinculin binds to talin and α -catenin in FAs and AJs, respectively, determining the ability of these supramolecular structures to bear force. In spite of these similarities, vinculin is phosphorylated in different sites when it is located in AJs or FAs.^[94] Interestingly, the degree of phosphorylation also determines force transmission in both kinds of adhesion.^[94,95] Another well studied component of FAs that also regulates cell–cell adhesion is FAK: it phosphorylates β -catenin producing VE-cadherin dissociation in a mechanically independent manner.^[96] On the other hand, when FAK is activated in cells seeded on stiff substrates, there is an increase in the expression of N-cadherin in mouse embryonic fibroblasts (MEFs)^[97] and vascular smooth muscle cells (VSMCs), suggesting that FAK can regulate the adhesive crosstalk in a tension-dependent and -independent way.^[91]

One of the main convergence blocks in the adhesive crosstalk are Rho GTPases, which act upstream and downstream of cadherin and integrin interactions. Rho GTPases regulate the assembly of FAs.^[98] Similarly, the assembly of AJs is Rho-, Rac1-, and Cdc42-dependent^[99] in a time- and cadherin-dependent way.^[100] Ouyang et al. showed that N-cadherin junctions inhibited Rac1 activity via phosphoinositide 3-kinase (PI3K). To perform these experiments, MEFs were seeded on fibronectin-coated micropatterned strips. They observed that N-cadherin inhibited Rac1 activity at the junction through the N-cadherin–p120-catenin axis,

whereas N-cadherin– β -catenin increased myosin II at the junctions. N-cadherin was also found to locally suppress integrins, creating subcellular polarization. Moreover, they observed Rac1 activation via integrins at the free end of the cells, suggesting integrin mediation in Rac polarity (Figure 2, right cell).^[101] Linked to these results, it has been seen that p120-catenin can also associate with E-cadherin to prevent the activation of integrin functions via Rap1.^[102]

New integrin adhesions activate Rho and ROCK, by increasing cell–matrix adhesion, cell shape, and cytoskeletal tension.^[103] These activations play an important role in cell–cell adhesion. As shown by Playford et al. in 2008, AJ formation can be modulated by Rho GTPases downstream of integrin signaling. They found that the inhibition of FAK caused the loss of epithelial morphology due to an increased activity of RhoA that inhibited cell–cell contacts.^[104] The same effect was observed by Sahai and Marshall: the activation of ROCK via RhoA promoted the disruption of cell junctions at AJs. On the other hand, the same study also observed that Rho signaling through Dia maintains AJs due to its ability to promote actin polymerization, which restores E-cadherin and α -catenin localization at the junction (Figure 2, right cell).^[105] It is evident that Rho GTPases are widely used by the cells as an intracellular communication mechanism which is influenced by both integrins and cadherins in a spatiotemporally controlled manner.^[5]

YAP is also one of the signaling proteins shared by different pathways initiated by both integrins and cadherins. The Hippo pathway keeps YAP in the cytosol. On the other hand, the mechanosensitive pathway promotes YAP translocation to the nucleus when cells are located in a stiff environment. By contrast, if cells are in softer substrates, YAP remains in the cytoplasm.^[63,106] Due to these changes in YAP location in a mechanical-dependent manner, YAP is named “mechanical rheostat.”^[107] Cosgrove et al. demonstrated that the crosstalk between integrins and N-cadherins produces changes in the mechanical state of the cells, leading to a decrease in YAP translocation to the nucleus when they are located in stiff environments.^[108] Finally, Barry et al. showed that cadherins by themselves can also be upstream regulators for integrins: mechanically stimulated VE-cadherin adhesions led to an increase in integrin activity.^[200] This regulation of integrins by cadherins was reported also for N-cadherin in zebrafish, where N-cadherins were observed to stabilize inactive α 5 integrin complexes close to cell–cell adhesions.^[110]

5.2. Effects on Cellular Functions

Signaling through cadherins, integrins, and their crosstalk has a crucial impact on cellular functions such as morphogenesis, migration, or differentiation. The process of tissue morphogenesis consists of cell assembly into tissues and large-scale collective movements, which are determined by the different cadherins and integrins presented within the developing tissues. During all the stages of development, there is a differential expression of the various types of cadherins, which act in distinct developmental contexts.^[111] Also, the communication between integrins and cadherins is regulated in a spatial, temporal, and tissue-specific way, which is crucial for morphogenesis and

collective cell migration.^[112] For example, during mesenchymal development, most interactions are cadherin-mediated within the cell-rich mesenchymal condensate, but, as development progresses, these interactions are restricted by ECM deposits secreted by differentiated progenitors that stimulate contact with the cell microenvironment.^[113,114] The communication between adhesion molecules is bidirectional: cadherin adhesions induce integrin-dependent fibronectin assembly, which is, for example, required for normal morphogenetic movements in early embryogenesis.^[109,115] During morphogenesis, the assembly of cells in epithelial sheets is essential for compartmentalization in the body and, at the same time, functionality of the epithelium is dependent on the polarization of the cells that create it. This polarization consists of a segregation of the cells into adhesive interactions with the ECM and a nonadhesive part facing a luminal space.^[116] Cadherin role is crucial in polarization and assembly in epithelial tissues, as shown in several reports.^[111,117,118] Also, the positioning of the apical surface of the epithelial sheets is controlled by contact of the cell surface with the ECM.^[119]

Mechanosensing through integrins and cadherins is necessary for the assembly and stability of epithelial tissues. It has been seen that adhesion to ECM proteins, such as fibronectin, stabilizes the epithelial state,^[120] but also promotes cell scattering due to increased RhoA and myosin light chain kinase activity, which disrupts cadherin-based adhesions.^[121] On the other hand, the stabilization of the epithelial state is produced by a stabilization of cell–cell adhesion induced by integrins,^[122] suggesting that AJs can respond differently to cytoskeletal tension induced by integrins: moderate contraction promote AJ stability but stronger tensions result in a disruption of AJs.^[123] Cadherin contacts also regulate ECM signaling. E-cadherin signaling through Scr and Rap1 promotes engagement of integrins to a basal ECM,^[124] although it locally suppresses integrin adhesion in apical junctions contributing to polarization.^[109]

Cell migration can also be impacted by the adhesive crosstalk. Indeed, during collective migration, the cells' cytoskeletons undergo an extensive reorganization, while being coupled between neighboring cells to allow migration in sheets, strands, and clusters. Three main characteristics define collective cell migration: physical and functional connection between the cells during movements, multicellular polarity, which organizes the actin cytoskeleton to generate traction and protrusions, and structural modification of the tissue along the path of the migrating cells. In order to remain connected, cells create AJs. Moreover, as mentioned above, cell–cell and cell–ECM interactions are in charge of the polarization,^[125] which creates “follower” and “leader” cells. These two kinds of cells have different morphologies which are mediated by the cytoskeletal organization, controlled by Rho GTPases and myosin II.^[126] In epithelial cell migration, the loss of E-cadherin contacts promotes weakened cell junctions and cell detachment, provoking a scattering of the cells that will follow a single-cell mode of migration. This is known as epithelial to mesenchymal transition (EMT).^[127] In EMT, the interaction between ECM–integrins and neighboring cells is crucial, because an enhancement in ECM contacts produced by an increase in ECM stiffness can produce disruption of cell–cell adhesion^[121] and promote cell scattering.^[128]

Another major cellular process that can be impacted by the adhesive crosstalk is cell differentiation. Differentiation is influ-

enced by cell adhesion receptors, ECM composition, cell density, and cell shape. For example, osteogenesis of human mesenchymal stem cells (hMSCs) on stiff matrices is guided by an interplay between FAK and RhoA/ROCK signaling, due to the implication of RhoA/ROCK in cell tension and myosin contractility; this promotes runt-related transcription factor 2 (RUNX2) activity, eventually leading to an osteogenic fate.^[129] FA formation and maturation are also known to promote osteogenesis.^[130] On the other hand, if the activity of RhoA is modulated, the lineage commitment can be changed: indeed, reducing the activity of this protein promotes adipogenesis. Osteogenesis has also been found to be regulated by N-cadherin, although these studies have shown differing effects. For example, Xu et al. found that N-cadherin negatively regulated MSC osteogenesis due to a negative regulation of β -catenin and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways.^[131] On the other hand, hyaluronic acid hydrogels functionalized with N-cadherin mimetic peptides were found to enhance hMSC osteogenesis.^[132]

In the case of chondrogenesis, a dramatic change in cell shape is observed during early differentiation stages compared to osteogenic cells. While in osteogenesis there is a flattening of the cells due to their interaction with fibronectin in the ECM, in chondrogenesis (as in adipogenesis) cells remain rounded.^[133] Moreover, YAP has been demonstrated to be a negative regulator of chondrogenic differentiation.^[134] These findings correlate with the observation that stiff matrices promote an osteogenic phenotype, due to the involvement of mechanosensing proteins which regulate YAP localization, such as FAK or Rho GTPases.^[135] On the other hand, the Hippo pathway influences chondrogenesis, whereas an overexpression of LATS1 correlates with the maintenance of the chondrogenic phenotype in vitro^[136].

6. Biomaterials That Mimic Cell–Cell Interactions

As highlighted in the previous sections, cell–cell adhesion plays a crucial role in several biological functions. For this reason, biomaterials have been widely used to study how cells interact with each other via cadherins and how this interaction can be harnessed for applications in regenerative medicine. The advantage of using biomaterials is that they can be engineered to provide signals that recapitulate cell–cell interactions, providing a platform to gain a deeper understanding of a myriad of cellular processes. Moreover, biomaterials allow to investigate the synergy between the spatiotemporal presentation of the ligands and other properties of the environment, such as dimensionality, structural features, or mechanical properties (e.g., mobility or stiffness).

There are several approaches to promote and investigate cell–cell interactions via biomaterial design: cadherin-based biomaterials make direct use of cadherins (or specific fragments) and cadherin-adhesion peptides (**Table 1**), while other approaches rely on careful design of materials functionalized with ECM proteins (or peptides) to control cell–cell adhesions. The use of mimetic peptides has gathered increasing interest due to their higher stability, easier biomaterial fabrication, and more precise control over chemical composition compared to the use of full proteins or protein fragments. Results obtained in various studies suggest that both functionalization with cadherins or with mimetic peptides, by chemically binding them to the

Table 1. Examples of biomaterials that use cadherin-based constructs.

Cadherin, fragment, peptide	Material type	Cell type	Biological effect	Ref.
N-cad-Fc	Alginate hydrogels	MSCs	Guided formation of neural networks	[139]
N-cad-Fc	Polystyrene plates	Embryonal carcinoma cells	Maintenance of undifferentiated state	[140]
E-cad-Fc	Antioxidative cell culture dishes	MSCs	Increase adhesion	[141]
E-cad-Fc	Glass	CHO cells	E-cad-mediated actin assembly	[142]
VE-cad-Fc	Polystyrene plates	Human umbilical vein endothelial cells (HUVECs)	Promote vascularization	[143]
N-cad EC1-5, EC1-2, HAVDI	Polyacrylamide hydrogels	MSCs	Paracrine effects	[138]
HAVDI	Hyaluronic acid hydrogels	MSCs	Chondrogenic regulation	[144]
HAV	Peptide nanofibers	MSCs	Chondrogenic differentiation	[145]
HAV	Au surfaces	Madin-Darby Canine Kidney (MDCK)	Cell adhesion and clustering	[146]
HAVDI	Nano- and microporous hydrogels	MSCs	Paracrine control	[147]

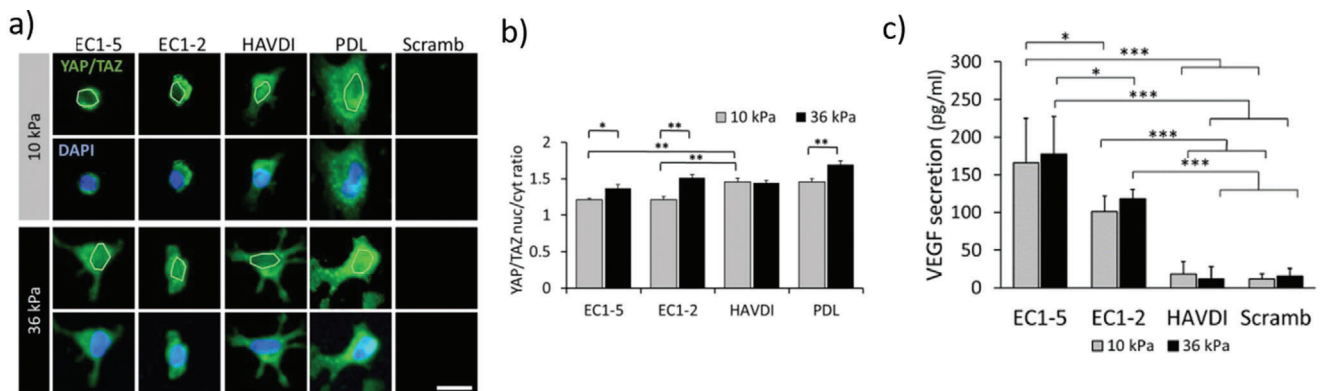


Figure 3. Effects of different N-cadherin constructs on MSCs. a) Representative images of YAP/TAZ (green) and DAPI (blue) on hydrogels with the different N-cadherin constructs. b) Quantification of YAP/TAZ ratios of the conditions showed in (a). c) Secretion of vascular endothelial growth factor (VEGF) by MSCs seeded on hydrogels functionalized with N-cadherin constructs. Reproduced with permission.^[138] Copyright 2020, Elsevier.

material, are able to support cadherin-mediated adhesions, although differences have been observed when comparing the effects of different cadherin fragments. For example, in the case of E-cadherin, constructs containing only the first and second ectodomains were not sufficient to mediate cell adhesion and spreading in the absence of integrin-based adhesions, allowing only single cadherin–cadherin adhesions without cadherin clustering.^[137] The effect of different N-cadherin constructs has also been recently studied. Specifically, the whole cadherin extracellular region, the first two ectodomains, and the HAVDI adhesion peptide were compared in terms of MSC behavior: differences in the constructs resulted in different MSC responses with regard to cell adhesion, YAP/TAZ translocation, paracrine secretion, and cell differentiation. Although MSCs showed similar spreading and traction forces when interacting with the different fragments, the N-cadherin extracellular domain showed stiffness-dependent changes in nuclear YAP/TAZ (Figure 3a,b), an increased paracrine secretion (Figure 3c), and myogenic differentiation compared to the HAVDI adhesion peptide. All these differences should be considered when selecting the fragment used for biomaterial design.^[138]

6.1. Biomaterials to Investigate Cell–Cell Adhesion

Due to the difficulty in isolating interactions in native cell–cell contacts, creating a biomaterial that dissects the effect of cadherin ligation alone is crucial to understand how cadherin adhesion affects cell behavior.^[148] Engineered surfaces have been one of the first biomaterials used to control and study cell response to cadherins.

In 2000, Lambert et al. created a model system to dissect the molecular mechanisms of cadherin-based adhesion and subsequent signal transduction to the cytoskeleton. Dimers of N-cadherin-Fc chimera immobilized on 6.2 μm latex beads were found to allow calcium-dependent bead–cell binding through N-cadherins. These experiments revealed that N-cad-coated beads recruited N-cadherin, α -, β -, and p120-catenins at the bead–cell contact sites, triggering the activation of tyrosine kinases as well as actin filament redistribution. This study showed for the first time that cell architecture, contractility, and microtubule network are essential for cadherin adhesion (the requirement of linkage to actin filaments was previously documented^[149]). It also proved that N-cadherin ectodomains

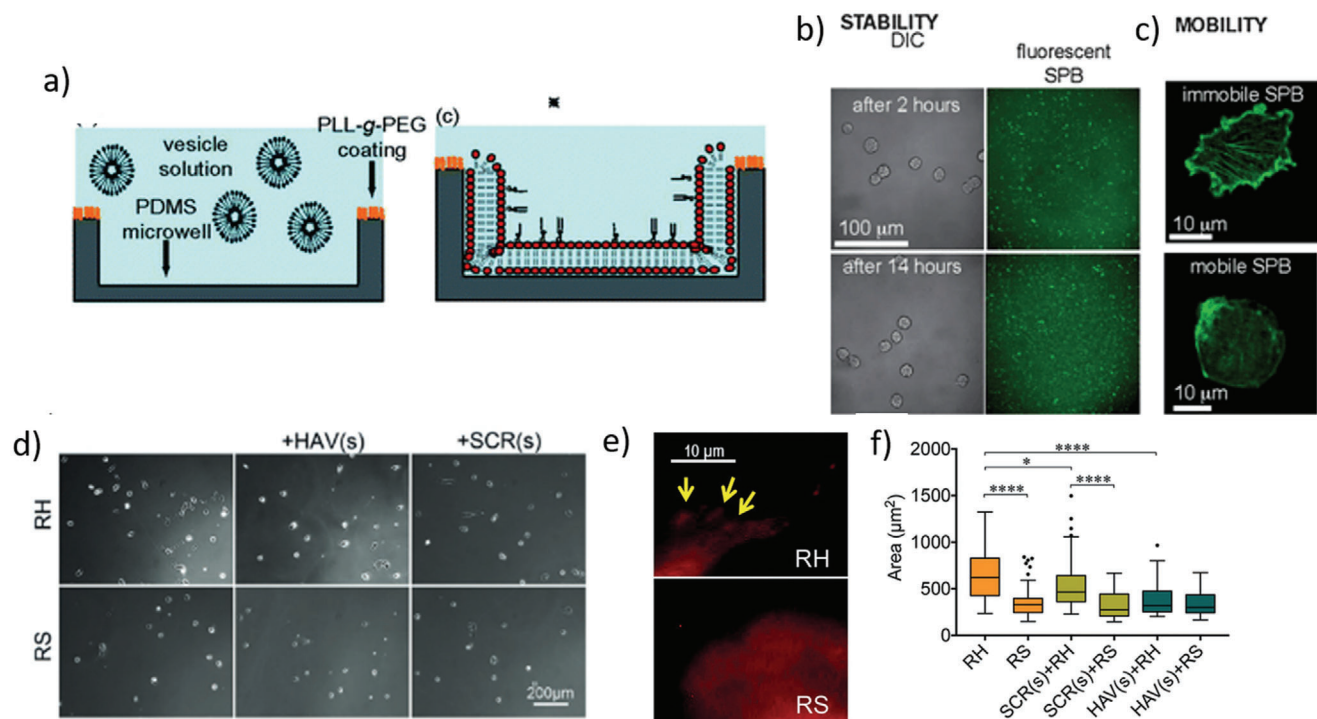


Figure 4. a) Schematic representation of lipid-coated microwells. b) Fluorescently labeled and cadherin-functionalized bilayers imaged after seeding and after 14 h to test the stability. c) Influence of ligand mobility on cell behavior. Adapted with permission.^[152] Copyright 2011, The Royal Society of Chemistry. d) MDCK adhesion at 6 h of cells seeded on RGD/HAV (RH) surfaces and RGD/SCR (RS) surfaces before and after the addition of free HAV or SCR peptides [(s) stands for soluble]. e) Images of cells stained for vinculin, showing the difference in FA. f) Area of MDCK cells cultured in the different conditions for 6 h. Adapted with permission.^[146] Copyright 2019, Wiley-VCH GmbH.

are sufficient to mimic cadherin-mediated cell contact and signal transduction.^[150] Similarly, Kovacs et al. coated glass surfaces with E-cad-Fc to determine the relationship between cadherin binding and cytoskeletal activity during early cadherin adhesive contacts: they observed that the homophilic ligation between the cadherin chimera and cellular E-cadherin activated the Arp2/3 complex, producing actin nucleation at the junction.^[142]

To fully understand how cells respond to cadherin-mediated adhesions, it is however necessary to develop biomaterials that present cadherins in a way that resembles how they are presented by the cells themselves. As previously described, cadherins are transmembrane proteins and hence are laterally mobile due to the viscous nature of the cell membrane. Biomaterials can be engineered to present controlled substrate mobility, allowing to study how this physical property affects cadherin-mediated adhesion. To achieve this, Tsai and Kam used lipid bilayers. Silica beads of 5 µm diameter were functionalized with the extracellular recognition domains of E-cadherin, either by directly adsorbing the fragments onto the beads' surface, or by attaching the cadherin to a lipid bilayer, resulting in a laterally immobile or mobile presentation of the cadherin. Lateral mobility of the E-cadherin was found to increase its recognition at lower concentrations, by promoting Rac1 activity. These results proved the mechanosensitivity of cadherin-mediated adhesions, and demonstrated the need of a proper biomaterial design when using cadherins.^[151] Andreasson-Ochsner et al. added another cue, dimensionality, enabling a more physiological-like 3D interaction of the cells with the cadherins. Specifically, they developed a 10 µm deep microw-

ell platform that presented a cell membrane mimetic interface made of supported lipid bilayers with tunable lateral mobility, functionalized with E-cad-Fc. CHO cells seeded on microwells with high cadherin lateral mobility showed a more diffuse actin organization and a better adaptation to the shape of the microwell due to their inability to apply forces (Figure 4a–c).^[152] Ge et al. also mimicked the cell surface by developing polymer-tethered lipid multi-bilayer stacks functionalized with N-cadherin linkers. In this system, another physical cue, stiffness, can be modulated depending on the number of stacked bilayers, and the dynamic assembly and disassembly of cadherin linkers into clusters normally observed at cell–cell interfaces can be replicated. Changes in cytoskeleton organization, AJ formation, and cellular traction forces were observed as consequence of bilayer stacking, demonstrating the role of stiffness in cell–cell adhesion.^[153]

Another platform that has been used to understand cell response to cadherin-mediated adhesion are hydrogels, polymeric materials that have a distinct 3D structure, tunable mechanical properties, and can be easily functionalized; these characteristics make hydrogels suitable platforms to study the interplay between cadherin engagement and a variety of structural or physical cues. An example of hydrogels that modulate cell–cell adhesions without direct cadherin functionalization was showed by Wang et al. In their study, polyethylene glycol diacrylate (PEGDA)-based hydrogels functionalized with gelatin or matrigel were used to investigate how stiffness affects cellular heterogeneity during proliferation from single cells to multicellular populations. Epithelial cells located on soft

substrates (1.2 kPa) had stronger E-cadherin expression and more E-cadherin/ β -catenin membrane localization compared to cells seeded on rigid coverslips. This study demonstrated that substrate stiffness regulates epithelial cellular heterogeneity by modulating E-cadherin/ β -catenin mechanotransduction.^[154]

6.2. Biomaterials to Investigate the Effects of the Adhesive Crosstalk

As elucidated in Section 5, cell adhesion occurs both via cell–cell and cell–ECM interactions. These two types of adhesions are connected to each other via the adhesive crosstalk, which regulates a wide range of cellular functions.

Li et al. used biomaterials to study the role of the adhesive crosstalk in the adhesion and clustering of epithelial cells. To investigate cell response to cell–cell and cell–ECM adhesions, MDCK cells were seeded on gold surfaces functionalized with different adhesion peptides: HAV (for E-cadherins) and RGD (for integrins), or a scrambled (SCR) cadherin control and RGD. Surfaces were functionalized in a 1:400 RGD to HAV/scrambled peptides ratio in order to allow enough adhesion without masking HAV-induced interactions. Cells were found to be more spread and to develop more mature FAs when seeded on RGD/HAV surfaces compared to RGD/scrambled surfaces. Addition of free HAV peptide in the culture medium dramatically decreased cell area, making it comparable to the cells seeded on RGD/scrambled surfaces. The reduction in the cell area was explained by the interaction of free HAV with E-cadherin on the cell membrane, decreasing E-cadherin participation in cell adhesion to the substrate. Conversely, adding free HAV to the RGD/scrambled surfaces did not change cell area (Figure 4d–f). This confirmed the cooperation of immobilized HAV–E-cadherin interaction with the integrin adhesion. The fact that only immobilized HAV was able to generate cell spreading demonstrated that HAV-induced cell adhesion was related to mechanosensing and to the ability to generate force. Indeed, the use of a contractility inhibitor, blebbistatin, abrogated cell spreading on RGD/HAV surfaces, while it did not have any effect on cells seeded on RGD/scrambled surfaces. Moreover, cell clusters were weaker on RGD/HAV surfaces due to a shift from cell–cell interactions to cell–substrate interactions.^[146]

Biomaterials have been also used to investigate the influence of the mechanical properties of the substrate and of the spatial organization of the receptors in the adhesive crosstalk. Polydimethylsiloxane (PDMS) substrates with different stiffnesses (5 and 60 kPa) were patterned with islands of fibronectin surrounded by E-cad-Fc. These substrates supported the formation of FAs when the stiffness was high, while both FAs and cadherin-mediated adhesions were observed when the stiffness decreased, showing an inhibition of cell–cell mimetic contacts on more rigid substrates.^[155] Stiffness also modulates intercellular force transduction mediated by VE-cadherins in endothelial monolayers, as shown by Andresen Eguiluz et al.^[156] Using polyacrylamide gels (1.1 and 40 kPa) functionalized with fibronectin, the authors observed that VE-cadherin-mediated cell stiffening in the monolayer was dependent on substrate stiffness. Specifically, VE-cadherins were mechanically stimulated via magnetic twisting cytometry to activate cell stiffening, showing higher relative stiff-

ening in soft substrates compared to stiff hydrogels and glass. This could be explained by the lower basal stress in soft gels, which offers a wider range of mechanical response. Moreover, the mechanical perturbation of VE-cadherins caused a decrease of cell contact area between cells on glass, and a higher increase in the gap area on stiffer matrices. Interestingly, force loading in VE-cadherin triggered cell–matrix junction remodeling by increasing the size and number of FAs only in stiff substrates and glass. These results show that although integrins were activated by intracellular signals, FA response and overall stiffening depend on outside mechanosensing,^[156] as observed in E-cadherin force transduction in single cells.^[157] Ultimately, this study shows that substrate stiffness modulates force-activated endothelial disruption, suggesting that ECM stiffening, for example, due to age, can contribute to endothelial junction destabilization.^[156] Lampi et al. also studied the influence of matrix stiffness on endothelial monolayers by using methacrylated hyaluronic acid hydrogels functionalized with RGD and photopatterned to create both soft and stiff microregions. While cells grew preferentially on stiff matrix regions during monolayer formation, VE-cadherin staining revealed disruption in cell–cell junctions and intercellular gaps located at stiffness interfaces, providing evidence that stiffness heterogeneity compromises the integrity of an endothelial monolayer.^[158]

Mechanotransduction via integrins has also effects on N-cadherins. Indeed, N-cadherin expression was found to be stimulated by ECM stiffness in VSMCs and MEFs. Higher matrix stiffnesses induced cell stiffening via Rho GTPases and subsequent activation of N-cadherin. In fact, when Rac1 activity was inhibited, N-cadherin expression was reduced. Upstream of Rac, FAK was found to be responsible for N-cadherin expression, connecting cell–ECM to cell–cell adhesion. Interestingly, N-cadherin was found to be an essential effector in cell cycling, both in vitro and in vivo, leading to cell proliferation. This mechanism is used by VSMCs, as the tissue stiffness increases in response to vascular injury. This was demonstrated by Mui et al., who showed that when cell spreading is limited, N-cadherin mediates cell proliferation via the FAK–p130Cas–Rac pathway.^[97] Although cadherins have been known to inhibit cell proliferation,^[159] these data, together with other reports,^[160] suggest that the proliferative capacity of cadherins can be modulated by the environment. Another study was presented by Cosgrove et al., who seeded MSCs on hyaluronic acid hydrogels with varying stiffnesses. These gels were functionalized with constant concentrations of RGD and varying amounts of HAVDI. Results showed that N-cadherin ligation reduced the mechanical state of the cell and consequently YAP/TAZ translocation to the nuclei, resulting in an altered interpretation of ECM stiffness. In this case, HAVDI ligation inhibited Rac1, which is in charge of the accumulation of myosin IIA in FAs, inhibiting their maturation and growth. This mechanobiological mechanism shows that the ligands presented to MSCs can alter the mechanosensing of the environment and, ultimately, cell fate.^[108]

6.3. Applications of Cadherin-Modified Biomaterials

Biomaterials are not only used to study biological paradigms; due to their unique chemical and physical characteristics, they have

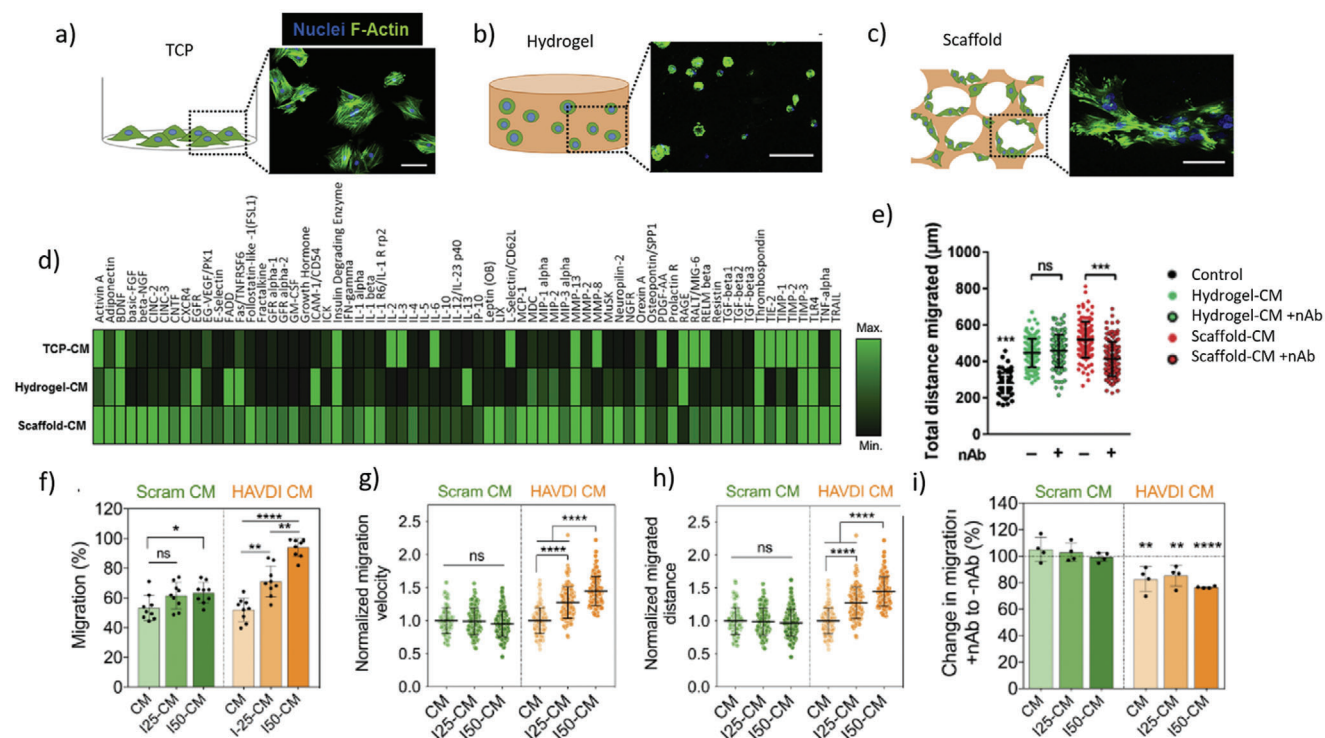


Figure 5. Effect of cadherin mimetic peptides in paracrine secretion. a–c) Schemes representing cells on tissue culture plates (a), nanoporous hydrogels (referred to as hydrogels) (b), and microporous hydrogels (referred to as scaffolds) (c). d) Heatmap showing the secretion profile of hMSCs on the different surfaces. e) Effects of conditioned media (CM) on myoblast migration with and without N-cadherin inhibition.^[163] f) CM from HAVDI-functionalized hydrogels stimulated myoblast migration even at low doses, while the maximum dose was needed for scram hydrogels. g) Migration velocity and h) migrated distance increased in HAVDI–CM stimulated myoblasts, while no change was observed in scram–CM stimulated myoblasts. i) Effects of N-cadherin blocking of MSCs cultured in HAVDI or scram hydrogels.^[147] Reproduced with permission.^[147,163] Copyright 2017, 2019, Elsevier.

been widely used for specific applications in tissue engineering, such as controlling paracrine secretion, stemness maintenance, or cell differentiation.

For example, biomaterials have been designed to enhance paracrine signaling. This is highly relevant in tissue engineering due to the various biological processes that can be influenced, such as cell migration, or differentiation.^[161,162] Indeed, the microstructure of hydrogels can be engineered to passively promote cell–cell interactions: microporous hydrogels promoted cell clustering, sensitizing hMSCs to growth factors and in turn affecting paracrine secretion activity. To prove that these effects were caused by N-cadherin-mediated adhesion, cells were seeded in tissue culture plates and encapsulated in nanoporous (10 nm) and microporous (125 µm) gels that inhibited and promoted cell–cell contacts, respectively (Figure 5a–c). The paracrine activity of cells seeded in nanoporous gels remained the same compared with cells seeded on a tissue culture plate. Nevertheless, when cells were seeded in the microporous scaffolds, a higher secretory profile was observed (Figure 5d). Indeed, when cell–cell contacts were inhibited by a blocking antibody, downregulation in the secretion of all cytokines produced by MSCs seeded in microporous gels was observed. The effects of the MSCs–conditioned media (CM) were tested on myoblast behavior, showing enhanced myoblast migration in microporous gels, an effect that was abrogated by N-cadherin blocking (Figure 5e).^[163] The same group further clarified the role of N-cadherin in paracrine

secretion by functionalizing their nanoporous gels with HAVDI. In this case, MSCs showed higher paracrine activity compared to MSCs seeded in hydrogels functionalized with a scrambled peptide (scram hydrogel) (Figure 5f–i).^[147] This HAVDI-mediated enhanced effect is presented as a solution to the lower cytokine secretion in nanoporous hydrogels. Other studies confirmed the importance of cell–cell contacts in stem cell secretory characteristics. For example, distinct porous environments formed by microgels with different diameters were created to investigate their influence in hMSC clustering and paracrine secretion. Consistent with the previously described study, microgels that allowed cell clustering showed higher MSC secretion. Moreover, if the hydrogels were functionalized with HAVDI, secretion was observed to increase even in the nanoporous hydrogels, demonstrating the importance of N-cadherin in paracrine function,^[164] and the crucial role of the cell–cell interactions in guiding cell response.

Another area in which cadherin-functionalized biomaterials have been useful is the expansion of pluripotent stem cells (PSCs), due to their potential in regenerative medicine and cell therapy. These cells require expansion in cell colonies to maintain cell–cell contacts and retain their pluripotency, nevertheless this expansion method limits scalability and without E-cadherin adhesion, apoptosis occurs. To solve these issues, alginate hydrogels functionalized with E-cadherin-mimicking peptides have been used by Richardson et al. Several E-cadherin adhesion peptides were employed (HAV10, Ala-Asp-Tyr (ADT) 10, HAV6, and

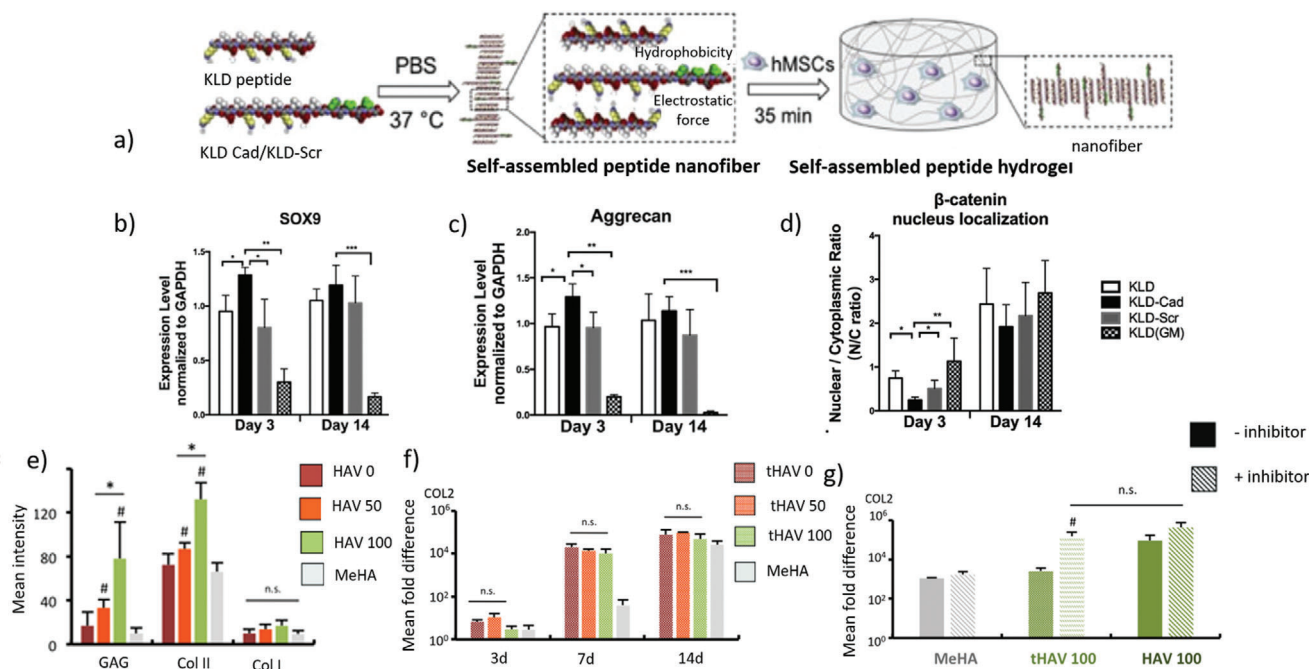


Figure 6. Cadherin-functionalized hydrogel effects on chondrogenesis. a) Scheme of the system developed by Li et al. b) Expression of SOX9 and c) aggrecan in hMSCs seeded in hydrogels without functionalization (KLD) or functionalized with HAVDI or scrambled peptide and nonchondrogenic group (GM). d) β -catenin localization in the different hydrogels. Reproduced with permission.^[167] Copyright 2017, Elsevier. e) Effect of HAV concentration in MSC expression of chondrogenic markers; the numbers next to HAV makes reference to the percentage of HAV peptides to the total number of peptides in the hydrogel (HAV + scrambled). f) Effect of the presence of transient HAV (tHAV) in the expression of collagen II (COL2). g) Effect of A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) inhibition in the expression of COL2. Adapted with permission.^[144] Copyright 2018, Wiley-VCH GmbH.

ADT6), based on the active domains of the bulge (ADT) and groove (HAV) regions of E-cadherin. Short and long variants (6 vs 10 amino acids) were used due to the influence of the length of the peptide on cell adhesion. All these peptides supported PSC adhesion, pluripotency maintenance, and differentiation potential, with HAV10 and ADT10 being more effective, probably due to their longer amino acid chain. This study highlights the importance of E-cadherin adhesion in PSC expansion and shows how adhesion peptides can be useful in regenerative medicine.^[165]

Cadherin-functionalized materials have also been used to guide cell fate. In this context, E-cadherin-coated poly(lactic-co-glycolic acid) (PLGA) microparticles induced hepatic differentiation of hMSCs in hMSC/E-cad-PLGA cellular aggregates. A 1:3 ratio of E-cad-PLGA to hMSCs was found to enhance the expression of hepatic-specific markers and achieve hepatic differentiation of MSCs after 4 weeks.^[166] N-cadherin or its adhesion peptides have instead been widely used in biomaterial design for their chondrogenic potential. For example, Li et al. in 2017 developed self-assembled N-cadherin mimetic peptide hydrogels that promoted chondrogenesis. To create these hydrogels, HAVDI was combined with the self-assembling peptide KLD-12 (Figure 6a). hMSCs encapsulated in the resulting gels had higher expression of chondrogenic markers such as SRY-Box Transcription Factor 9 (SOX9) or aggrecan (Figure 6b,c) as well as higher matrix deposition compared to nonfunctionalized or scrambled hydrogels. Further analysis revealed that N-cadherin adhesion promotes chondrogenesis by upregulating the expression of glycogen synthase kinase 3 beta (GSK-3 β), which in-

creases β -catenin degradation (Figure 6d). The degradation of β -catenin reduces its translocation to the nucleus, inhibiting the Wnt/ β -catenin signaling pathway.^[167] This pathway is considered to be a major modulator in osteogenesis and chondrogenesis via the translocation of β -catenin to the nucleus, enhancing osteogenesis via RUNX2 upregulation^[168] and suppressing chondrogenesis via SOX9 downregulation.^[169] Aggrecanase-degradable PEG-based multiacrylate gels functionalized with HAVDI were also used to induce chondrogenesis; they promoted cell-cell interactions in hMSCs and osteochondral repair after being implanted in rabbits for 18 weeks.^[170] The efficacy of HAV-functionalized hydrogels in vivo has been also explored using injectable thermosensitive 3D hydrogels. These scaffolds benefit from host-guest interactions between β -cyclodextrin, linked to a thermosensitive hydrogel, and adamantane, which was functionalized with transforming growth factor beta 1 (TGF- β 1) and HAV. The thermosensitivity of the hydrogel allows its injection in a sol state and its gelation at 37 °C. The gels were injected into pockets of living animals, producing chondrogenesis.^[171] In another study, N-cadherin stimulation to promote chondrogenesis was achieved by functionalizing alginate hydrogels with a peptide derived from the low-density lipoprotein-receptor-related protein 5, which also binds N-cadherin.^[172] Finally, Cimenci et al. proposed a peptide hydrogel made of nanofibers with HAV residues. The fibers promoted viability and cell adhesion and enhanced the expression of cartilage matrix components.^[145]

How cadherin adhesion peptides are temporally presented also influences chondrogenic differentiation. This effect was studied

using hyaluronic acid hydrogels that encapsulate MSCs: the gels were functionalized either with HAV adhesion peptides in different doses (referred to as “HAV” conditions) or with an ADAM10-cleavable domain between the hydrogel and the different doses of HAV (referred to as “tHAV” conditions). Endogenous ADAM10 on the MSCs cleaved the peptides, reducing HAV presentation within the gels in a time-dependent fashion. Stable HAV presentation enhanced chondrogenesis in a dose-dependent manner, in such a way that chondrogenic markers were the greatest for higher concentrations of HAV (Figure 6e). Conversely, transient presentation via addition of the ADAM10-cleavable domain abrogated the increase in chondrogenesis markers and matrix production (Figure 6f). ADAM10 inhibition enhanced again MSC differentiation (Figure 6g)^[144] by stabilizing HAV, revealing the importance of time and dose presentation of HAVDI in chondrogenic differentiation. All these material platforms prove the ability of biomaterials that stimulate stable N-cadherin-mediated adhesion and signaling in guiding cell fate toward chondrogenesis, providing exciting solutions for cartilage repair.

Hydrogels functionalized with N-cadherin peptides have also been used for osteogenic differentiation. Hyaluronic acid hydrogels functionalized with RGD and HAVDI promoted osteogenesis by mimicking the pro-osteogenic niche in the endosteal space.^[132] It is known that N-cadherin promotes osteogenesis in early stages of differentiation,^[173] while maintained presentation inhibits the canonical Wnt/ β -catenin, inhibiting mature osteoblast formation.^[168] It is thought that due to the low seeding density and slow proliferation of hMSCs seeded on hyaluronic acid gels, N-cadherin contact is limited, promoting osteogenic differentiation. Also, the N-cadherin adhesion peptide interaction with osteoblasts recreated the pro-osteogenic niche environment, contributing to hMSC osteogenesis. As this hydrogel degraded over time, overinteraction with N-cadherin did not occur. Interestingly, addition of free HAVDI to the gel reduced the osteogenic activity, due to its antagonistic effect with the tethered peptide. These gels were also functionalized with RGD, whose interaction with integrins is also important for osteogenic differentiation.^[174] For this reason, the implication of N-cadherin alone in osteogenic differentiation cannot be concluded.^[132] Another study showing the effects of N-cadherin in periosteal cell differentiation was presented by Evans et al., who used lipid bilayers functionalized with the receptor. Their work proved the relevance of N-cadherin presentation in short-term cell behavior. Indeed, it was seen that N-cadherin on lipid bilayers promoted cell aggregation; on the other hand, cells on bilayers without N-cadherin remained nonadherent but with increased N-cadherin transcription and zonula occludens-1 (ZO-1) (a tight junction membrane protein) expression. These platforms placed periosteal cells in distinct cellular contexts, which guide the emergence of tissue architecture.^[175]

N-cadherin signaling has another important role in neural regeneration. PEG hydrogels functionalized with a gradient of HAVDI peptides were used in the neural differentiation of murine embryonic stem cells. Cells showed a loss of pluripotency at higher concentrations of HAVDI, with an increase in neurite extension length, although the percentage of polarized cells remained unaffected. However, the highest concentration of N-cadherin adhesion peptide (537×10^{-6} M) also increased apoptotic markers.^[176] The same material was used to study

the effect of HAVDI gradients on the neural differentiation of induced pluripotent stem cells, showing similar concentration-dependent effects on survival, neurite extension, and neural differentiation.^[177]

Finally, VE-cadherin-functionalized biomaterials have been used to guide hMSCs toward endothelial differentiation. Gao et al. used a polyamidoamine/thiolated hyaluronic acid injectable hydrogel functionalized with hVE-cad-Fc to promote adhesion, proliferation, and upregulation of the expression of endogenous VE-cadherin and secretion of growth factors in hMSCs. These effects were achieved due to an overexpression of VE-cadherin compared to nonfunctionalized hydrogels, which led to secretion of proangiogenic-related factors. Moreover, the activation of mechanotransductive VE-cadherin signaling pathways led to the phosphorylation of FAK and to the cytoplasmic sequestration of YAP;^[178] previous studies have showed that phosphorylated YAP is able to bind to α -catenin and induce angiogenic activation in endothelial cells.^[179] With the same aim of promoting endothelial differentiation, Nie et al. created a chitosan hydrogel that instead of using direct VE-cadherin contacts, promoted its expression via nitric oxide (NO) release. The NO released from the hydrogel upregulated early endothelial cells markers and VE-cadherin expression in mouse embryonic stem cells.^[180] These two platforms show different approaches to endothelial differentiation without the use of exogenous growth factors, representing a safe cell therapy source for vascular diseases.

Another emerging field of research that could take advantage of cadherin-based biomaterials is organoid engineering. Indeed, due to the importance of cell–cell adhesion in development and multicellular tissues, controlled cadherin engagement is crucial in the design of organoid systems. This engagement can be regulated either by designing the structural, adhesive, and degradation properties of the organoid matrix to permit cadherin-mediated adhesions or by introducing synthetic cadherin interactions, as the ones reviewed in this Section 6.^[181] While many organoid systems are still based on matrigel, which provides a less controlled and chemically undefined environment, the use of ad hoc functionalized materials could better promote the formation and maturation of the organoids. Nevertheless, this is a still emerging field, and most of the research on organoids supported by engineered materials is still focused on using cell–ECM binding ligands.^[182–184]

6.4. Applications of the Adhesive Crosstalk in Biomaterial Design

As mentioned in Section 5.2, one of the cellular functions controlled by the adhesive crosstalk is collective migration. Several biomaterials have been designed to fully understand and replicate this fundamental biological process. For example, Borghi et al. in 2010 investigated the role of the spatial organization of integrin- and cadherin-based adhesions during migration by seeding individual cells on micropatterned polyacrylamide surfaces functionalized with collagen IV and E-cadherin in alternating stripes. FAs were created only in collagen areas and cell migration only occurred if collagen was present; purely E-cadherin-functionalized surfaces instead did not support migration. Moreover, an increase in the concentration of E-cadherin in the combined surfaces did not affect the migration rate. Cell migration

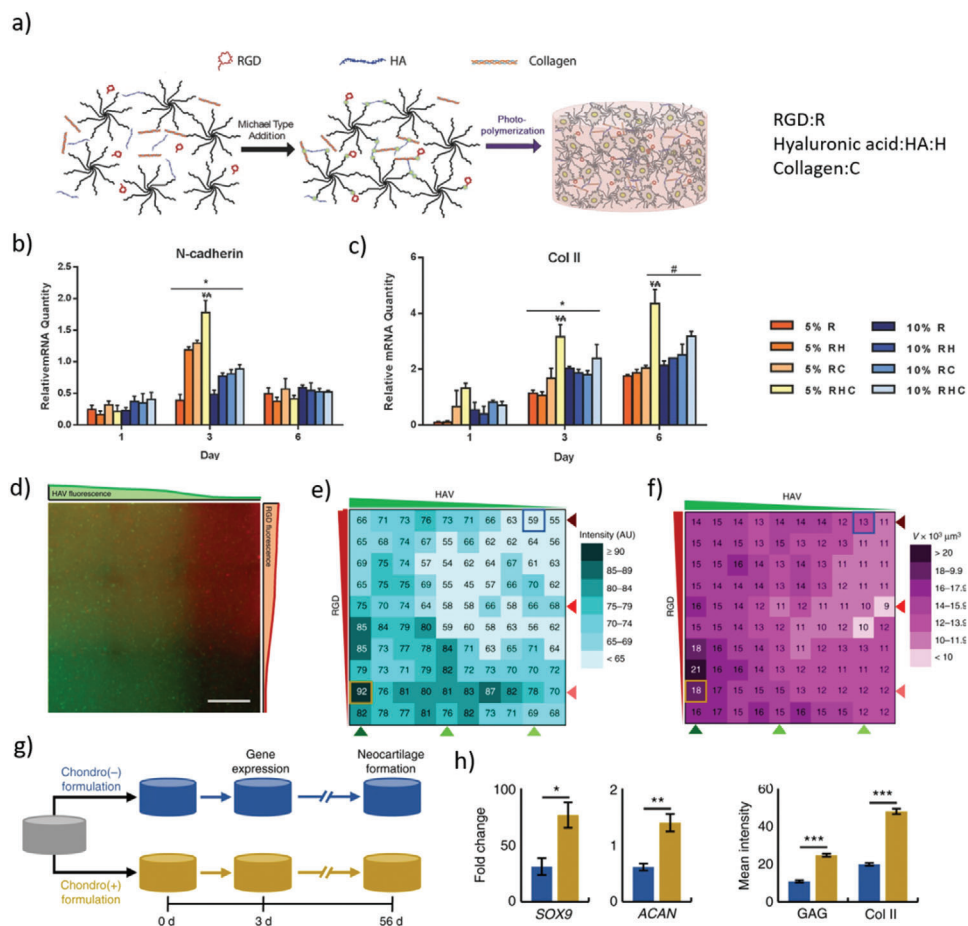


Figure 7. a) Scheme of the hydrogels designed by Carrion et al. b,c) Expression of N-cadherin (b) and collagen II (c) in MSCs seeded on hydrogels with different chemicals (RGD, hyaluronic acid, and/or collagen) and mechanical (5% and 10% PEG) properties. Adapted with permission.^[187] Copyright 2016, Wiley-VCH GmbH. d) Image of the peptide gradients in combinatorial hyaluronic acid gels. Differential expression of e) SOX9 (blue) and f) aggrecan (purple) in the combinatorial gels. g) Hydrogels with specific chondro (+) and chondro (-) formulations. h) Expression of chondrogenic markers in hMSCs seeded in chondro (+) or chondro (-). Reproduced under the terms of the CC-BY 4.0 International License.^[188] Copyright 2018, the Authors. Published by Springer Nature.

was found to be influenced by the direction of the stripes, due to the distribution of traction forces where FAs were created. Since E-cadherin-mediated adhesion controlled the location of FAs by allowing their formation only on ECM-coated regions, cell–cell adhesion emerged as a tool to control migration directionality.^[185] The same group then created a minimal tissue mimic platform that simulates the 3D interactions that the cells experiment in the process of wound healing, introducing dimensionality in the material platform. This interface consisted of a basal ECM surface functionalized with ECM proteins and an orthogonal wall functionalized with E-cadherin. MDCK cells were seeded on the platform, ensuring their separation from the E-cadherin wall by using an obstacle. When this barrier was removed, the cells migrated toward the wall, stopping when they made contact with it. This indicated that 3D E-cadherin adhesion is a minimum stop signal sufficient to induce self-healing in healing epithelia. Indeed, when the cells got in contact with the E-cadherin wall, polarity and migration were disrupted and junctions with the wall were created. On the other hand, when cells approached an inert wall used as control, F-actin was still present, indicating that the

polarity remained.^[186] These results, compared to the alternated stripes, where E-cadherin presentation did not lead to a reduction in migration rate, highlight the importance of the 3D spatial presentation of adhesive ligands in collective migration.

As mentioned in the previous section, hydrogels functionalized with cadherins are widely used for chondrogenic differentiation. Several groups have harnessed the potential of the adhesive crosstalk to achieve the same effect on cell fate, either by functionalizing hydrogels with ECM adhesion molecules and cell–cell peptides, or by using ECM cues alone to drive cell clustering. An example of the latter strategy is the hydrogel system developed by Carrion et al. (Figure 7a): hydrogels containing RGD, hyaluronic acid, and type-I collagen induced cell aggregation by stimulating N-cadherin expression. Moreover, the highest expression levels of N-cadherin and other chondrogenic markers were observed in softer gels, highlighting the importance of matrix stiffness in chondrogenesis (Figure 7b,c).^[187] This study represents a remarkable example of use of the adhesive crosstalk for regenerative purposes without the need for direct cadherin functionalization of the biomaterial. On the other hand,

hyaluronic acid hydrogels gels functionalized with both RGD and HAVDI peptides have also been engineered to promote chondrogenesis: MSCs were encapsulated within the gels and the effect on chondrogenesis of varying concentrations of these two ligands was studied. The hydrogels were manufactured with orthogonal gradients of both peptides, creating a scalable platform that allows the screening of different chemical signals (Figure 7d). The authors found higher expression of chondrogenic markers in regions with high HAVDI and low RGD concentrations (Figure 7e,f). These results were validated by encapsulating cells in hydrogels with specific concentrations of both peptides: a chondro (+) condition with high HAVDI and low RGD and a chondro (−) condition with opposite concentrations of both peptides. Again, the highest concentrations of chondrogenic markers both after 3 and 56 days in culture were shown by chondro (+) hydrogel, indicating that combinatorial hydrogels can be used to screen cell–material interactions (Figure 7g,h).^[188]

Another screening platform used to present arrays of different adhesive peptides at varying ratios and spacings was developed using a block copolymer that self-assembles into cylindrical nanodomains.^[189–193] Specifically, a panel of adhesion peptides characteristic of chondrogenesis or osteogenesis was used to drive MSC differentiation toward three chondrocyte phenotypes (transient, persistent, and hypertrophic). The adhesion peptides mimicked N-cadherin, E-cadherin, collagen I, II, and III, decorin, heparin binding, fibronectin, and laminin. By selecting the conditions that supported spontaneous aggregation of hMSCs and cartilage matrix deposition, the authors found that while E-cadherin is necessary for chondrogenic differentiation, long-term presentation of N-cadherin induced a transient chondrogenic phenotype. Of the ECM components, heparin was observed to induce hypertrophic chondrocytes, collagen I to promote osteogenesis, while decorin and collagen II adhesion peptides induced a persistent chondrogenic phenotype.^[194]

The temporal regulation of the adhesive crosstalk is also important, as mentioned in Section 5. While in literature there are cadherin-mimetic materials that allow a temporal regulation of the presentation HAVDI (as seen in Section 6.3), and advanced substrates that provide a temporally controlled presentation of ECM ligands already have a more widespread use,^[195–197] to the best of our knowledge, cadherin- and ECM-functionalized materials that permit this kind of dynamic regulation for both ligands have not been engineered yet. A possible approach to directly present two independently regulated ligands can be designed using living materials, which incorporate engineered bacteria that express proteins in an inducible manner.^[198] Currently, an indirect strategy to achieve this temporal regulation of both cell–cell and cell–ECM interactions can be via tuning of the biodegradation of the substrate, in a way that, for example, allows for it to be remodeled by cells to progressively facilitate cadherin engagement.^[199]

7. Outlook

In this review, we have discussed the interplay between the signaling pathways activated by cell–cell and cell–ECM adhesions, as well as the biomaterials developed in recent years to elucidate these interactions and harness them for applications that span from paracrine secretion to cell differentiation.

While integrin and cadherin signaling pathways converge at multiple points, some of the shared molecules execute site-specific functions depending on their upstream and downstream effectors, and the bidirectional nature of these signaling events makes their study very complex. This interplay among varying intracellular machineries guides the spatial organization inside the cells, ultimately driving cell behaviors such as cell migration, cell differentiation, or ECM remodeling. Moreover, the mechanosensitive nature of cadherin- and integrin-mediated adhesions implies that these pathways are influenced by the mechanical properties of their surroundings.

Due to the crucial biological effects of the adhesive crosstalk, an increasing number of studies have tried to elucidate different parts of the signaling network or to exploit the current knowledge to control cell behavior. Nevertheless, within native tissues, these cadherin- and integrin-based complexes are part of a much wider network that comprises multiple points of adhesions, and several cell types. Hence, the challenge still remains to fully understand the adhesive crosstalk in its multiple contextualities. Despite these issues, the biomaterials presented in this review have demonstrated their effectiveness in controlling the spatial presentation of cadherins and integrins, for example, via 2D patterning or 3D ligand presentation, and in elucidating the interplay between ligand presentation and other environmental cues, including structural features or mechanical properties, in a bid to further approximate native physiological surroundings. These biomaterials eventually succeeded in guiding cell behavior toward specific goals, such as the control of paracrine secretion, guided cell migration, or controlled cell differentiation.

Ultimately, the studies reported in this review show not only that biomaterials are crucial to improve our understanding of the molecular mechanisms underpinning complex biological phenomena, but also that the design of future biomaterials should be driven by this knowledge. When engineering new biomaterials, the role of cell–cell adhesions should not be overlooked, and should instead be an integral part of the design of a material-driven approach for a successful manipulation of cell fate.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

adhesive crosstalk, biomaterials, cadherins, integrins, mechanosensing

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