Synthetic Hydrogels Mimicking Basement Membrane Matrices to Promote Cell-Matrix Interactions

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Naturally-derived materials have been extensively used as 3D cellular matrices as their inherent bioactivity makes them suitable for the study of many cellular processes. Nevertheless, lot-to-lot variability, inability to decouple biochemical and biophysical properties and, in some types, their tumor-derived nature limits their translational potential and reliability. One innovative approach to overcome these limitations has focused on incorporating bioactivity into cytocompatible, synthetic hydrogels that present tunable physicochemical properties. This review provides an overview of successful approaches to convey basement membrane-like bioactivity into 3D artificial hydrogel matrices in order to recapitulate cellular responses to native matrices. Recent advances involving biofunctionalization of synthetic hydrogels via incorporation of bioactive motifs that promote cell-matrix interactions and cell-directed matrix degradation will be discussed. This review highlights how the tunable physicochemical properties of biofunctionalized synthetic hydrogel matrices can be exploited to study the separate contributions of biochemical and biophysical matrix properties to different cellular processes.

1. Introduction

Cell-extracellular matrix (ECM) interactions transduce mechanical and biochemical signals that regulate diverse cellular processes during embryogenesis, tissue repair, homeostasis, and pathogenesis (Martins-Green and Bissell, 1995; Lukashev and Werb, 1998; Midwood et al., 2004; Streuli, 1999). Bissell, Mostov and others have pioneered the use of 3D collagen and laminin (i.e., Matrigel™) gels in organotypic cultures that recreate morphogenetic developmental programs observed in the organism (Barcellos-Hoff, 1989; O'Brien et al., 2001; Montesano et al., 1991). Similarly, an understanding of cell-ECM interactions has been a major goal for biomaterials science in order to engineer synthetic materials

that can recapitulate complex ECM-mediated cellular responses. Although cytocompatible synthetic materials exhibit the advantage of tunable biophysical and biochemical properties, the adaptation of these materials as biologically active cellular matrices remains a challenge. Nevertheless, recent studies have demonstrated major advances in the development of engineered synthetic hydrogel matrices that can mimic ECM properties, specifically, but not limited to, basement membrane (BM) characteristics. These research advances address three fundamental questions of biomaterial science: (1) How can we integrate suitable biochemical cues into synthetic hydrogels that mimic cell-BM interactions? (2) Is it possible to recapitulate native cellular programs by tuning the physicochemical properties of synthetic hydrogel matrices? (3) What benefits do these engineered synthetic hydrogels provide over natural matrices?

1.1 Basement Membrane

The basement membrane (BM) is a specialized form of the ECM that is mainly composed of a mixture of glycoproteins (Kalluri, 2003). It is found adjacent to most epithelial and endothelial layers in the body as it serves as a structural support that separates cell monolayers from the underlying connective tissue. BM also dictates many cellular processes by specific interactions between BM components and cellular receptors (LeBleu et al., 2007).

The four major components of the BM are: type IV collagen, laminin, nidogen (formerly called entactin), and perlecan (Kalluri, 2003; LeBleu et al., 2007; Yurchenko, 2011). Each major component plays a different role in the structural assembly of the matrix as well as in its bioactivity (Yurchenko, 2011). The BM structure is characterized by self-assembly of type IV collagen and laminin into polymeric networks that provide structural stability. Type IV collagen is a non-fibrous collagen that forms a triple helix composed of three α -chains, while laminin is the most abundant non-collagenous protein in the BM and its structure is characterized by a heterotrimeric three-pronged fork made of α -, β -, and γ -chains (LeBleu et al., 2007). At least twelve different laminin isoforms have been identified, resulting from the combination of five α -, three β -, and three γ -chains (Siler et al., 2000; Engbring et al., 2003). Laminin isoforms provide different architectural variations to the BM that in turn create a functional diversity that contributes to BM tissue-specificity (Cheng et al., 1997; Kalluri, 2003). Moreover, laminin and type IV collagen contribute to matrix bioactivity by containing specific motifs that are primarily recognized by cell receptors such as integrins (Kalluri, 2003). Interactions between the $\alpha\beta$ integrin

heterodimers and ECM peptide motifs mediate essential cellular processes such as cell migration, proliferation, and differentiation (Hynes, 2009; Huttenlocher and Horwitz, 2011; Hynes, 2014; Garcia, 2005) (Figure 1a). Furthermore, the type IV collagen and laminin networks are linked together by nidogen and perlecan, which are secreted by cells as single molecules and cannot self-assemble into a network (LeBleu et al., 2007) (Figure 1b).

1.2 BM-like Natural 3D Matrices

In an attempt to create materials that mimic the native basement membrane environment, matrices consisting of extracts or purified proteins (e.g. type I collagen) have been developed. These 3D natural matrices have been used for a wide range of studies to model epithelial cell morphogenesis (Yu et al., 2005) and migration (Wolf et al., 2009), cancer invasion (Gaggioli et al., 2007) and metastasis (Nguyen-Ngoc et al., 2012), as well as many epithelial morphogenetic programs (Shamir and Ewald, 2014).

1.2.1 Matrigel™

Matrigel™ is the commercial name for a BM extract secreted by Engelbrecht-Holm-Swarm (EHS) mouse sarcoma cells. Matrigel™ is a complex mixture of over 1,000 proteins (Hughes et al., 2010). It primarily consists of type IV collagen, laminin and nidogen, which makes it the most BM-like natural matrix model (Hughes et al., 2010; Yurchenco, 2011). Epithelial morphogenesis (Peng et al., 2014) and oncogenesis (Debnath et al., 2003), as well as intestinal organoids generation (Sato and Clevers, 2013; Yui et al., 2012; Fatehullah et al., 2016) are current major areas that involve 3D cultures in Matrigel™. A study by Sato et al (2009) exemplifies the potential of Matrigel™ by establishing a 3D culture condition that generates intestinal crypt organoids from single intestinal stem cells in the absence of a cellular niche. After 4 days of encapsulation in Matrigel™, and supplemented with soluble proteins essential for crypt proliferation (R-spondin 1 and epidermal growth factor) and expansion (Noggin), the resulting multi-cellular structures comprise approximately 100 cells, which is consistent with the 12-hour cell cycle of proliferative crypt cells. Additionally, the presence of stem cells at the crypt bottom as well as four major differentiated epithelial cell types are present, demonstrating the ability of Matrigel™ to support intestinal epithelial expansion reminiscent of normal gut (Fatehullah et al., 2016).

1.2.2 Laminin

Laminins are highly biologically active molecules that can act as strong adhesive substrates for many cell types, and have been found to promote cell adhesion, migration, protease activity, proliferation, tumor growth, angiogenesis, and metastasis (Siler et al., 2000; Engbring et al., 2003; Colognato and Yurchenco, 2000). Among the more of **than** a dozen laminin isoforms, laminin-111 (also known as laminin-1; composed of α 1-, β 1-, and γ 1-chains) is the most widely used for 2D and 3D culture systems as it can be isolated from EHS mouse tumors and it is commercially available (Siler et al., 2000; Kikkawa et al., 2013). The use of laminin-111 as a culture substrate has helped identify specific peptide motifs that promote malignant phenotypes by increasing tumor adhesion and migration, as well as tumor cell metastasis through induction of protease production (Engbring et al., 2003; Kikkawa et al., 2013; Turpeenniemi-Hujanen et al., 1985). Although laminin-111 is the most prominent and studied form of laminin, studies have provided insights into the importance of other laminin isoforms in the morphogenesis of different epithelial organs (Ekblom et al., 1998; Li et al., 2015; Durbeej et al., 1996).

1.2.3 Other Naturally-derived 3D Matrices

Other naturally derived materials that have been used as cellular matrices include fibrin, alginate and hyaluronic acid gels. Fibrin gels, which are products of the polymerization of fibrinogen by the proteolytic activity of thrombin, started as one of the first biomaterials used to prevent bleeding and promote wound healing (Janmey et al., 2016), and transitioned to a variety of biomedical applications including islet transplantation (Riopel et al., 2015). Alginate and hyaluronic acid gels, which are derived from marine brown algae and vertebrate connective tissues, respectively (Matricardi et al., 2013), are polysaccharides that have been extensively used as biomaterials for controlled release of drugs and tissue engineering applications (Lee and Mooney, 2012; Kim et al., 2011; Lam et al., 2014).

1.3 Limitations of Natural Matrices

Although 3D natural matrices have been found suitable for the study of many cellular processes, they are limited by lot-to-lot compositional and structural variability (Hughes et al., 2010) which **decreases** their reliability. Additionally, these matrices are limited by the inability to decouple mechanical and biochemical properties. For instance, a common approach to vary the mechanical properties is to change the bulk concentration (e.g., increase matrix density). However, these changes in bulk

concentration inevitably alter other matrix properties, such as adhesive ligand density and fiber density/structure. Furthermore, as Matrigel™ is a tumor-derived matrix, its clinical translational potential is limited for regenerative medicine applications. For these reasons, approaches have been pursued to overcome these limitations, including the combination of purified natural BM proteins with biocompatible synthetic hydrogels that exhibit defined mechanical properties (Beck et al., 2013; Hutson et al., 2011). However, although these materials have decoupled mechanical and biochemical properties, they still have limited translational potential. Therefore, as the field of biomaterials continues to advance, novel engineered synthetic hydrogel matrices that present independently-tunable BM-like bioactivity and mechanical properties have been developed, overcoming each of the aforementioned limitations of natural matrices.

2. Engineered Synthetic Hydrogel Matrices

Hydrogels are water-swollen crosslinked polymer networks that can be considered synthetic equivalents of ECM matrices. Commonly used hydrogel polymers for biomedical applications include poly(acrylic acid) (PAA), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), polyacrylamide (PAAm), and polypeptides (Zhu, 2010). These mesh-like structures are held together by either covalent crosslinks or non-covalent interactions that include ionic interactions, hydrogen bonds, affinity and hydrophobic interactions, polymer crystallites, physical entanglements, or a combination of the above (Peppas et al., 2000).

2.1 Physical Properties of Hydrogels

Hydrogel stiffness and swelling behavior are two important physical characteristics that are inversely related and are primarily controlled by polymer density (Flory and Rehner, 1943; Anseth et al., 1995; Drury and Mooney, 2003). The swelling behavior of hydrogels involves a swelling force produced by the thermodynamic compatibility of the polymer chains and water, which is counterbalanced by the retractive forces exerted by its crosslinks (Flory and Rehner, 1943; Peppas et al., 2000). Swelling equilibrium is reached when these two forces are equal (Peppas et al., 2000). Consequently, a hydrogel with high (low) polymer density has a greater (smaller) number of crosslinks that cause increased (decreased) retractive forces that will result in less (more) swelling. Moreover, an opposite effect is followed in hydrogel stiffness, as polymer density is proportional to the matrix stiffness; a high polymer

density would produce a denser (thus, stiffer) polymer network. Hydrogel elastic modulus is an important physical parameter to consider in the design of a synthetic cellular matrix as many studies demonstrate matrix stiffness-dependent cellular responses, such as extent of cell migration and differentiation (Lutolf et al., 2003; Das et al., 2015).

2.2 Synthesis of Hydrogels

Among several methods to produce synthetic hydrogel matrices, photo-polymerization and chemical reaction crosslinking stand out as two of the most widely used for biomedical applications (Hoffman, 2002; Zhu, 2010). Photo-polymerization involves the crosslinking of two water-soluble polymers (Fig. 2a), or copolymerization between one or more monomers and one multi-functional monomer (Fig. 2b), via photo-ionization of the monomers using light, often in the UV range. The high reactivity of the photo-ionized polymer components induces crosslinking reactions that produce a crosslinked network. As the polymerization is light-dependent, this technique grants in situ spatiotemporal control over the mechanical properties of the hydrogel and presentation of incorporated ligands (Zhu, 2010; Kloxin et al., 2010; Nguyen and West, 2010; Lee et al., 2014).

Chemical reaction crosslinking involves a direct chemical reaction between linear or branched polymer macromolecules (macromers) with a di-functional or multi-functional crosslinking agent. Each agent, which has a smaller molecular weight, crosslinks two or more macromers together forming a mesh-like structured hydrogel (Hoffman, 2002) (Fig. 2c). Examples of chemical reactions used to produce hydrogels are chemical ligation (Hu et al., 2009), click chemistry (Polizzotti et al., 2008) and Michael-type addition reaction (Phelps et al., 2012).

These two general hydrogel synthesis methods have been widely used for generating artificial 3D cellular matrices as they allow easy incorporation of BM-like biochemical properties, which will be discussed in the next section. Although these synthesis techniques are based on covalently crosslinking the synthetic hydrogel constituents, there are other synthesis methods that involve crosslinking by non-covalent interactions that are common in naturally-derived hydrogels, such as alginate “ionotropic” hydrogels which are formed based on electrostatic interactions (Hoffman, 2002) (Figure 2d).

2.3 Incorporation of BM-like Properties

The BM contains key biophysical and biochemical characteristics that are essential for epithelial and endothelial cell attachment and viability as well as numerous cellular processes that include proliferation, differentiation, and migration. These cellular processes are primarily mediated by cell-matrix interactions via integrins, the ability of cells to remodel their supporting matrix via degradation of their microenvironment, and by presentation of growth factors (Lu et al., 2011). Consequently, in order to incorporate bioactivity into artificial hydrogel matrices, successful approaches have focused on incorporating bioactive peptides derived from natural ECM. Three common themes explored in biofunctional hydrogels are (1) cell adhesive peptides, (2) protease-degradable crosslinkers, and (3) growth factor-binding domains in order to mimic native cell-BM interactions (Fig. 2e).

2.3.1 Cell Adhesive Peptides

Integrin binding to short peptide sequences present in BM proteins such as collagen and laminin provides cellular attachment and triggers signals that direct cell function, cell-cycle progression and expression of differentiated phenotypes (Hynes, 2002; Giancotti and Ruoslahti, 1999; Danen and Sonnenberg, 2003; Danen, 2005). Therefore, incorporating biofunctional short peptide sequences into 3D artificial hydrogel systems has been an effective method to promote integrin-mediated cellular functions. These peptide motifs have the advantage of being relatively stable, and they can be covalently tethered to the hydrogel polymer backbone at tunable densities via integration of reactive groups, yielding a biofunctionalized hydrogel (Zhu, 2010). The most widely used cell-adhesive motif is the short peptide arginine-glycine-aspartic acid (RGD); this oligopeptide was originally identified in fibronectin but is also present in many other ECM proteins including laminin and collagen (Ruoslahti, 2003). Presentation of RGD on synthetic matrices supports cell adhesion by targeting integrins, such as $\alpha_v\beta_3$. Many studies have demonstrated successful BM-like bioactivity of RGD-functionalized hydrogels, as compared to non-functionalized matrices, by directing essential cellular processes such as attachment and spreading (Schmedlen et al., 2002), migration and invasion (Puperi et al., 2015; Halstenberg et al., 2002), and stem cell support (Nuttelman et al., 2005) and differentiation (Saha et al., 2007). Furthermore, other peptides like IKVAV and YIGSR, which are derived from laminin, can also be incorporated into hydrogels together

with RGD to orchestrate ligand density-dependent signal presentation independently of the mechanical characteristics of the matrix (Saha et al., 2007; Fittkau et al., 2005). This demonstrates the potential of engineered hydrogels as matrices that can elucidate tunable biochemical signals that direct complex cellular processes via presentation of cell adhesion peptides.

2.3.2 Protease-Degradable Crosslinkers

The capacity of cells to modify their microenvironment via matrix degradation is essential for their ability to migrate and proliferate, as well as for tissue remodeling and homeostasis (Lu et al., 2011). ECM remodeling involves degradation and modification of its protein components, most significantly by cell-secreted or membrane-bound matrix metalloproteinases (MMPs) (Sternlicht and Werb, 2001). Therefore, crosslinking hydrogel matrices with MMP-cleavable peptides has been a widely used strategy to allow for cell-directed modifications of the matrix biophysical characteristics. These short peptide sequences are derived from ECM proteins, such as type I or type IV collagen, and incorporation into synthetic hydrogel matrices conveys sensitivity to MMP-dependent degradation (Lutolf et al., 2003; Mann et al., 2001). To incorporate these MMP-cleavable peptides within synthetic hydrogel networks, the peptides are encoded to contain groups that react with the base macromer. For example, a linear MMP-cleavable peptide containing N- and C-terminal cysteines (with free thiols) will react with maleimide-functionalized PEG macromers via a Michael-type addition reaction (Phelps et al., 2012) (Figure 2c, e). Moreover, studies show that mutations to, or combinations of, different MMP-sensitive crosslinking peptides can yield hydrogels with different degradability rates that result in variations of cellular responses within the hydrogel, such as extent of cell invasion (Lutolf et al., 2003) and determination of stem cell fate (Das et al., 2015; Rape et al., 2015). Such control over the degradability rate of hydrogels may facilitate the design of synthetic ECM-like materials that present high to low MMP sensitivity that is necessary for short- to long-term matrix stability, respectively. Thus, this demonstrates that artificial hydrogel matrices with tunable degradability rates present BM-like bioactivity that can direct a wide range of cellular responses independent of their cell adhesive peptide density.

2.3.3 Growth Factor-binding Domains

The BM hosts a wide variety of signaling molecules such as growth factors (GFs), which reside in the matrix by non-covalent interactions with heparan sulfate proteoglycans such as perlecan and other

minor BM components (agrin and type XVIII collagen) (Guvendiren and Burdick, 2013; Halfter et al., 1998). GFs play significant roles in tissue development by eliciting a variety of essential cellular responses such as cell proliferation, stem cell differentiation, and vascular and organ morphogenesis (Kleinman et al., 2003). Therefore, presentation of specific GF types and densities to cells through the use of artificial hydrogel matrices has been an effective approach to orchestrate desired cellular responses. The traditional method for GF incorporation into hydrogels is direct encapsulation, which involves physical entrapment of GFs within the matrix which will be released as the hydrogel degrades and/or will diffuse out of the hydrogel network (Sokic and Papavasiliou, 2012). Nevertheless, as this method may not provide long-term control over GF presentation, another approach has been to covalently tether GFs to the hydrogel backbone (Guvendiren and Burdick, 2013; DeLong et al., 2004). However, as covalent linkage may compromise GF activity by alterations to its conformation or masking its active sites, strategies focus on incorporating GF-binding domains into hydrogel matrices by covalently tethering heparan sulfate-containing molecules to the polymer backbone that interact with GFs (Martino et al., 2013; Sakiyama-Elbert and Hubbell, 1999; Hudalla and Murphy, 2011). This method, compared to previous approaches, has gained popularity as it mimics native BM-GF non-covalent interactions within artificial hydrogel matrices. Additionally, this strategy facilitates the design of hydrogels that exhibit temporal control over GF sequestration and presentation by functionalization of the polymer backbone with heparan sulfate-containing molecules that exhibit different affinity levels for different GF types (Martino et al., 2013). Consequently, investigations have demonstrated inherent GF-directed cellular responses via presentation of GF-binding domains within synthetic hydrogels, such as in vivo neovascularization (Jha et al., 2015; Cai et al., 2005) and wound healing (Martino et al., 2013).

3. Applications of BM-mimicking Hydrogels

As the fields of cell and matrix biology progress, our understanding of what biological cues are essential for specific cellular processes becomes clearer, and thus, more robust synthetic hydrogels that present these essential biological signals have been engineered. In order to construct such materials, it is critical to understand how changes in the chemical and physical characteristics of hydrogels translate into changes in the local microenvironment of encapsulated cells. The establishment of an artificial hydrogel microenvironment that directs epithelial morphogenesis, controls cell fate, and provides insights into

cancer progression are among the most recent advances in the field of biomaterials, and various studies are highlighted below.

3.1 Epithelial Morphogenesis

Epithelial morphogenesis is part of the organogenesis program of multicellular organisms where the epithelia form transient structures, such as tubules and hollow spherical systems (cysts), that further develop into more complex organs (Martin-Belmonte and Mostov, 2008). During this process, epithelial cells establish tight junctions among neighboring epithelial cells through their lateral membranes, and connections to their supporting BM via integrin receptors in their basal membranes (O'Brien et al., 2002), which contribute to their distinctive cellular polarity (O'Brien et al., 2002; Peng et al., 2014). Studies using 3D collagen and laminin gel cultures have demonstrated that the epithelial morphogenesis developmental program is regulated by biochemical and mechanical signals that result from cell-BM interactions (O'Brien et al., 2002; Yu et al., 2005; Mroue and Bissell, 2013). In these studies, single-cell encapsulation of epithelial cells, such as MDCK cells, results in cell proliferation into a multicellular aggregate and further development of cysts composed of a polarized epithelial monolayer that recreates the morphogenesis of epithelial organs (McAteer et al., 1986). Although these natural matrices have been able to recapitulate the epithelial morphogenesis program of MDCK cells, they do not offer the capacity to study individual contributions of the mechanical and biochemical properties of the ECM towards epithelial morphogenesis. Therefore, Enemchukwu et al (2016) established an engineered ECM-mimetic PEG hydrogel system with independent control over ligand density and presentation, proteolytic degradation and mechanical properties to independently study the effects of ECM biochemical and biophysical properties on MDCK cell morphogenesis. In this study, normal cyst growth, polarization, and lumen formation were restricted to a narrow range of hydrogel elasticity that was controlled by the polymer density. Additionally, RGD density dramatically regulated apicobasal polarity and lumenogenesis independently of cell proliferation, and a threshold level of MMP-directed hydrogel degradation rate was required to regulate these critical epithelial characteristics. This study offers new insights into how ECM biochemical and biophysical properties independently regulate epithelial morphogenetic behaviors, as well as present a platform technology that could potentially be adopted in developmental cell and tumor biology fields to study ECM-directed processes.

3.2 3D iPSCs Generation

Since the discovery of induced pluripotent stem cells (iPSCs), reprogramming of somatic cells has been considered a multi-step process that is initiated by cytoskeletal and epigenetic alterations (Sancho-Martinez and Belmonte, 2013; Polo et al., 2012; Caiazzo et al., 2016). Some of these alterations are related to effects of the ECM on cells, for example, iPSC generation can be influenced by biophysical parameters in 2D culture (Downing et al., 2013). In order to better understand the role of the microenvironment on somatic-cell reprogramming, a synthetic 3D ECM-like hydrogel culture system that supports the initiation of reprogramming was developed by Lutolf (Caiazzo et al., 2016). These PEG hydrogels allow for precise control of the physiochemical characteristics of the cellular microenvironment which is not possible in 2D culture. In order to take full advantage of the tunable nature of this hydrogel, a high-throughput imaging system was used to detect pluripotency levels as a function of hydrogel stiffness, susceptibility to MMP degradation, and functionalization with different proteins previously shown to play a role in regulating pluripotency. This analysis yielded an engineered 3D PEG hydrogel that demonstrated a 2.5-fold higher reprogramming efficiency of human fibroblasts into iPSCs as compared to 2D culture. The accelerated reprogramming was attributed to hydrogel matrix selection of colony-forming iPSCs by limiting proliferation of non-colony-forming cells, and to the pronounced morphological changes that may cause the key events for the initiation of iPSC generation. These findings provide the first proof of principle for 3D reprogramming in synthetic matrices, and demonstrate the capacity of an engineered ECM-like hydrogel system to reveal the influence of the physiochemical characteristics of the cellular microenvironment in cell fate regulation.

3.3 Tumor Progression

Genetic changes in tumor cells and the biochemical and biophysical properties of the local microenvironment have been major attributions to cancer progression and metastasis (Hanahan and Weinberg, 2011; Bissell and Radisky, 2001; Provenzano et al., 2009). The effect of changes in the local microenvironment have been evaluated through 2D culture models and, although they have been contributory in the understanding of tumor progression, they do not recapitulate the complex properties of the 3D in vivo microenvironment (Bissell and Radisky, 2001; Nelson and Bissell, 2005). Consequently, Weaver, Bissell and others have done seminal work in studying how abnormalities in the ECM contribute

to tumor progression through the use of 3D collagen and laminin (i.e., Matrigel™) gels (Levental et al., 2009; Egeblad et al., 2010; Weaver et al., 1997; Lu et al., 2012). Whereas these naturally derived materials can recapitulate complex features of native ECM, results are often inconsistent and/or difficult to interpret due to batch-to-batch variability and undefined composition (Hughes et al., 2010). Therefore, in order to further comprehend the effects of the local microenvironment in tumor progression in a 3D culture while overcoming the principal limitations of naturally derived materials, Singh et al. (2015) developed an engineered PEG-based synthetic hydrogel system that presents a cell adhesive peptide motif (RGD) and MMP-degradable crosslinks that induce cell adhesion and cell-directed degradability. In this study, this ECM-like synthetic hydrogel served as a cellular niche that promoted the formation of human melanoma cell (WM239A) clusters from a single cell in a matrix stiffness-dependent manner. Furthermore, to study cancer cell migration and invasion, melanoma cell migration within the hydrogel system was studied in the presence of human dermal fibroblasts, which resulted in cell migration to surrounding regions in a way that resembled invasion of patient-derived melanoma xenograft tumors (Li et al., 2003; Hu et al., 2008). Taken together, these results showed the application of a synthetic 3D hydrogel culture system that models the growth, migration and invasion of a human melanoma cell line or xenograft tumors, and at the same time serves as a tool to study the role for several critical components of the microenvironment during tumor progression.

4. Outlook for BM-like Synthetic Hydrogel Matrices

Although the studies discussed in this review have developed novel artificial hydrogel matrices that present BM-like properties, several challenges need to be addressed in order to continue engineering synthetic matrices that recapitulate and direct complex cellular processes. For instance, there is a need for hydrogels that can recapitulate the *in vivo* microenvironment by presentation of multiple adhesive ligand types at specific densities that can orchestrate complex cellular responses. Such artificial matrix designs are important for the support of complex cellular systems (e.g. primary and stem cells) that still represent a challenge due to their increased sensitivity, and to further direct innate cellular functions. Furthermore, as different studies have demonstrated successful culture of complex cellular systems using Matrigel™, future developments of BM-like synthetic hydrogels should move towards designing hydrogels that recapitulate mechanical and structural (e.g., fibrillar structure) properties of Matrigel™ and collagen gels.

Consequently, designing artificial matrices that present combinations of biochemical signals by functionalization with multiple adhesive ligand types, and possess biophysical characteristics of Matrigel™, may lead to hydrogels that reiterate the in vivo microenvironment of complex cellular systems and can, thus, further expand their application as cell-delivery vehicles for in vivo studies. Therefore, as new ways of integrating bioactivity into hydrogel matrices are designed, these novel engineered synthetic materials will continue to offer new developments in regenerative medicine and tissue engineering fields, and promise innovative therapeutic options that naturally derived materials cannot provide.

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Figure Legends:

Figure 1: Three-dimensional arrangement of basement membrane components and its interaction with cellular receptors. Based on LeBleu et al., 2007 (A) BM-directed cellular response is primarily

initiated by interactions between integrin cell receptors and specific peptide motifs in BM proteins. (B) Three-dimensional arrangement of the four major basement membrane components.

Figure 2: Synthesis methods of engineered synthetic hydrogels. Photo-polymerization of two water-soluble polymers (A) and copolymerization of one monomer and one multi-functional monomer (B) via covalent reaction after UV irradiation. (C) Direct chemical reaction of a branched polymer with a di-functional crosslinking agent. (D) Electrostatic interactions of an anionic polymer with divalent cations yields an “ionotropic” hydrogel. (E) Encapsulated cells in an engineered biofunctionalized hydrogel exhibiting adhesive ligand, growth factor-binding domain and MMP-degradable crosslinks.

Figure 1:

A

B

Figure 2: