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REVIEW

Approaches in extracellular matrix engineering for determination of adhesion molecule mediated single cell function

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Abstract **The native extracellular matrix (ECM) and the cells that comprise human tissues are together engaged in a complex relationship; cells alter the composition and structure of the ECM to regulate the material and biologic properties of the surrounding environment while the composition and structure of the ECM modulates cellular processes that maintain healthy tissue and repair diseased tissue. This reciprocal relationship occurs via cell adhesion molecules (CAMs) such as integrins, selectins, cadherins and IgSF adhesion molecules. To study these cell-ECM interactions, researchers use two-dimensional substrates or three-dimensional matrices composed of native proteins or bioactive peptide sequences to study single cell function. While two-dimensional substrates provide valuable information about cell-ECM interactions, three-dimensional matrices more closely mimic the native ECM; cells cultured in three-dimensional matrices have demonstrated greater cell movement and increased integrin expression when compared to cells cultured on two-dimensional substrates. In this article we review a number of cellular processes (adhesion, motility, phagocytosis, differentiation and survival) and examine the cell adhesion molecules and ECM proteins (or bioactive peptide sequences) that mediate cell functionality.**

Keywords Extracellular matrix, integrins, biomaterials, natural polymers, peptide sequences, RGD

Introduction

The native extracellular matrix (ECM) is a stress-tolerant, interconnected network of macromolecules that not only defines the three-dimensional architecture of an organ but is also engaged in a complex relationship with the cellular elements that populate the surrounding environment. Cells alter the composition and structure of the ECM to regulate the material and biologic properties of the microenvironment; in turn, the chemical composition and structure of the ECM can modulate the onset and persistence of cellular processes (Simpson et al., 1994; Martins-Green, 1997). Bioactive domains, or peptide sequences, within native ECM proteins interact with cell surface receptors, playing a vital role in both day-to-day cellular activities as well as during wound healing and disease states. Communication between the cell and ECM molecules directs various cellular processes, such as adhesion, proliferation, differentiation, migration and apoptosis, as well as growth factor and cytokine modulation.

Significant research into cell-ECM adhesive interactions has been conducted using two-dimensional protein substrates; these results have provided important information on the influence of adhesion molecule mediated single cell function. However, three-dimensional ECM mimetics better approximate the cellular environment of native tissue and result in cell behavior and functionality that more closely reflects that seen *in vivo*. This paper will provide an overview of the methods used to analyze single cell function as regulated by adhesion molecule response to ECM proteins. In addition, we review methods by which engineered extracellular matrices can be utilized to analyze cell-ECM adhesion mediated interactions and their influence on cell processes. Finally, we suggest future directions for integration of molecular biology and materials science for applications in tissue engineering.

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Outside-in signals

"Outside-in signaling" is a term used to describe the ability of cells to use their adhesion molecules to integrate signals from their surroundings; cell surface membranes present a variety of cell adhesion molecules (CAMs) that attach and respond to other molecules within the external environment. By taking advantage of the fact that many of these adhesion molecules bind in a non-redundant and specific manner to ECM proteins, investigators have begun to elucidate the methods by which ECM proteins can modulate cell activity through adhesion molecule outside-in signaling. Cell adhesion proteins [which include cadherins, selectins, integrins and immunoglobulin superfamily (IgSF) CAMs] are typically composed of an intracellular, a transmembrane, and an extracellular domain. These molecules are responsible for the outside-in signaling that is critical for guiding cell functionality. All cells express a variety of adhesion molecules; for example, osteoblasts found in bone express high levels of integrins $\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_5$, low levels of integrins $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$ and E (epithelial)-cadherin and cadherin-11 (Anselme, 2000).

Cadherins are calcium dependent, homophilic (interacting only with CAMs of the same kind) cell-cell adhesion receptors (Fig. 1). There are many members of the cadherin family, such as E-cadherins, P-cadherins (placental), N-cadherins (neural), R-cadherins (retinal) and VE-cadherins (vascular-endothelial), though E-cadherins are the most widely studied (Gumbiner, 1996). Cadherin proteins are highly associated with embryonic development (controlling tissue separation, boundaries and shape) and are involved in regulation of rapidly growing tissues (such as the lining of the gut, epidermis, neuronal synapses and prevention of tumor cells) in adults (Gumbiner, 2005). The extracellular portion of most cadherins consists of five cadherin-type repeats (bound by Ca^{2+} ions) that form rod-like proteins, while the core complex is composed of p120 catenin (bound to the juxtamembrane region) and β-catenin which binds α-catenin (which binds actin and actin binding proteins). It appears that these catenins function as a direct physical link to actin and regulators of signaling molecules that influence the state of cytoskeleton and the adhesive state of the extracellular binding domain on the outside of the cell (Gumbiner, 2005).

Selectins are calcium dependent, heterophilic (they interact with other CAMs or the ECM) adhesion receptors (Fig. 1) (Varki, 1994). These proteins initiate a number of critical interactions among leukocytes (L-selectins), platelets (P-selectins) and endothelial cells (E-selectins and P-selectins) and are responsible for the low-affinity leukocyte rolling on endothelial cells. Each selectin contains a N-terminal C-type lectin domain, an epidermal growth factor (EGF)-like motif, short repeats similar to complement-regulatory proteins, a transmembrane domain and a short cytoplasmic tail. Selectins support interactions between leukocytes and the vascular endothelium; in Leukocyte Adhesion Deficiency II syndrome (LAD II), selectin ligands (typically carbohydrates on the cell surface) are absent, and, subsequently, neutrophil transmigration into sites of inflammation does not occur. Elevated levels of E-selectin are detected in arthritic joints, psoriasis and contact dermatitis and an increase in the expression of L-selectin is observed in patients with acquired immunodeficiency syndrome, leukemia and malignant tumors (Golias et al., 2011).

Integrins are calcium dependent, heterophilic (they interact with IgSF CAMs or the ECM) adhesion receptors (Fig. 1) (Golias et al., 2011). These proteins are composed of an α and

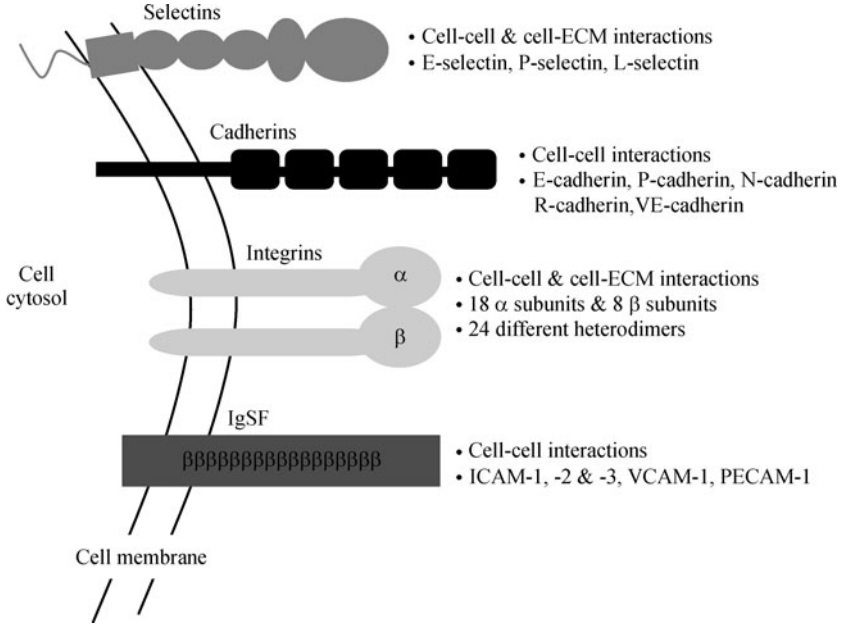

Figure 1 Overview of cell adhesion molecules.

β chain linked together through non-covalent bonds and maintain the integrity of the cytoskeletal-ECM linkage. The integrin family is composed of 18 α subunits and 8 β subunits that can combine to form 24 different heterodimers (Stupack and Cheresh, 2004). The α subunit determines integrin ligand specificity and is composed of a seven-bladed β propeller connected to a thigh, a calf-1 and a calf-2 domain. The β subunit connects to the cell cytoskeleton to influence a number of signaling pathways. It is comprised of a plexin-sempahorin-integrin (PSI) domain, a hybrid domain, a β1 domain and four cysteine-rich EGF repeats. Knockout animal models have provided significant information about the function and importance of integrins; for example, knockout of integrin α_8 results in absent or reduced kidneys, knockout of α_L, α_M, α_X and α_D results in reduced immune response and knockout of β_3 results in platelet defects (Barczyk et al., 2010).

The IgSF superfamily are highly abundant, non-calcium dependent homo- or heterophilic adhesion receptors that interact with other IgSF CAMs or integrins (Fig. 1). Their structure is characterized by repeated, tightly packed β strands. Many members of the IgSF family, such as intercellular adhesion molecules-1 and −2 (ICAM-1, ICAM-2), vascular adhesion molecule-1 (VCAM-1) and platelet-endothelial cell adhesion molecule-1 (PECAM-1), have been implicated in vascular disease. ICAM-1 is the most well studied of the IgSF family and is basally expressed on a variety of cell types; expression of this molecule is highly regulated on and promotes leukocyte adhesion to endothelial cells. The expression of ICAM-1 is extremely elevated in patients inflammatory disorders such as septic shock, LAD and with cancer (Golias et al., 2011). VCAM-1 is also highly expressed and regulated on the surface on the endothelium and mediates leukocyte adhesion to endothelial cells. This adhesion protein may also play a significant role in mediating the initiation of atherosclerosis (Cybulsky et al., 2001).

While integrins and selectins are the adhesion molecules primarily responsible for cell associations with the ECM, we include IgSF CAMs and cadherins in this review as adhesion molecules that are potentially altered in their expression and functionality as a result of selectin and integrin mediated outside-in signaling. Likewise, IgSF and cadherin mediated binding to cells and non-ECM molecules may influence integrin and selectin regulated cellular activity in response to natural matrix proteins in the surrounding microenvironment. In either case, the integration of cells with matrix proteins within the vasculature or extravascular space of any tissue is of paramount importance to a multitude of cellular processes.

Natural matrix proteins

Tissues of the human body are composed of cells and an extracellular matrix. Cells modulate bodily processes and perform tissue maintenance and regeneration (during wound healing or disease states) while the ECM, a group of macromolecules, acts as the main structural inhabitants to the tissue. ECM components consist of polysaccharide gels and natural polymers (collagen, elastin, etc.) that are consistently being secreted and modified by the cellular component of the ECM (Sell et al., 2010). Interactions between cell surface receptors and ligands found on ECM components result in cellular processes that maintain healthy tissue and heal or regenerate diseased or wounded tissue. Natural matrix proteins, including collagen, elastin, fibronectin and laminin, are highly distributed throughout the body and are logical choices for studying cell-ECM interactions and for use in matrix engineering. Additionally, a number of other naturally derived products (some not found in humans) have been evaluated for use as ECM components in engineered tissue. For example, significant research in bone tissue engineering has focused on the use of the mineral hydroxyapatite (HA) and the polysaccharide chitosan. HA is a naturally occurring mineral in human bones that has exhibited excellent mechanical properties, biocompatibility and osteoconductivity. Chitosan, a naturally occurring polymer in shellfish, has been shown to be biocompatibile and biodegradable, also supporting cellular ingrowth as a component of bone grafts (Ma et al., 2001; Bhattarai et al., 2005; Woo et al., 2007; Thein-Han and Misra, 2009; Wang et al., 2010; Barbani et al., 2011).

Among the most exciting demonstration of natural matrix proteins for use in adhesion molecule mediated tissue development are those conducted by Miller et al. The Niklason group has been investigating naturally derived polymers and acellular matrices for use as tissue engineered bronchioles and lungs. Using a bioreactor, they were able to produce a human bronchiole model composed of lung fibroblasts embedded in a collagen matrix surrounded by airway smooth muscle cells and bronchial epithelial cells (Miller et al., 2010). This model can be used to examine airway remodeling events that are associated with chronic respiratory disease. This group also cultured decellularized rat lungs with pulmonary epithelial cells and vascular endothelial cells together in a bioreactor; the resulting mechanical properties were similar to those in native lung tissue and the engineered lungs participated in gas exchange when implanted into rats for short time intervals (Petersen et al., 2010). Human skin substitutes are also being developed in a similar manner; Shepherd et al. (2006) decellularized the dermis of human cadavers and cultured the acellular matrix with keratinocytes and endothelial cells. These skin substitutes promoted graft vascularization even in animal models with vascular insufficiency. These models are pertinent examples of how tissue engineering scaffolds that allow for cell-ECM interactions can provide significant insight that may lead to the development of functional implants for organ replacement or tissue regeneration.

Collagen

Collagen, of which 28 types have been identified, is the most abundant protein in the body and acts as a major structural and biologic protein for both hard and soft tissues (Telemeco et al., 2005; Abraham et al., 2007). This protein is highly dynamic and continually undergoes remodeling by cells for physiologic functionality. An example of this remodeling is demonstrated in the transition between granulation tissue and scar formation during wound healing; wound fibroblasts degrade the provisional matrix (primarily using matrix metalloproteinases or MMPs), synthesize, and deposit a new collagenous matrix that is strengthened over time with the formation of collagen bundles and intermolecular cross-links (Singer and Clark, 1999). The most prominent collagens are types I, II, III, IV and V; types I, II and III are fibril forming collagens and constitute much of bone, cartilage, tendon, skin and muscle while types IV and V are non-fibrillar collagens and comprise the basal lamina and interstitial tissues, respectively (Table 1). In native tissues, collagens serve as both mechanical structural proteins as well as modulators of cellular activity; for example, in blood vessels type I collagen acts as a structural component (in the outermost layer) while type IV collagen regulates transmigratory activity (in the innermost layer).

Each collagen type is unique, yet all collagens are composed of a triple helical structure (three polypeptide α chains individually wound to the left but coiled together to the right) and contain 4-hydoxyproline as a distinct marker (Barnes et al., 2007). The reverse twist of the polypeptide chains and molecule as a whole contributes to the tensile strength of collagen and helps to resist lengthwise deformation (Bigi et al., 2004). Parameters that influence the configuration of the collagen protein (such as density, packing, degree/type of cross-linking and orientation) as well as the molecular variations among different types of collagen produce the varying mechanical properties (and functions) established in differing tissues (Cheema et al., 2011). The molecular organization differentiates the collagen types and is based on α1, α2 or α3 chain composition and the length of the resulting fibrils (for example, type I collagen is composed of two α1 chains and one α2 chain with fibrils 50 nm in diameter while type II collagen is composed of three α1 chains with fibrils 80 nm in diameter) (Sell et al., 2010). Cross-linking is tissue specific and the mechanical properties of fibril forming collagens (I, II, III, VI and XI) are dependent upon covalent cross-links (Ricard-Blum, 2011).

Denaturation of the collagen protein results in a breakdown of the collagen triple helix into random coils, altering bioactive domain presentation to cells and altering the biologic properties of the protein. Collagen denaturation occurs during tissue injury and remodeling when cells must degrade a provisional matrix in order to deposit a new one. This denaturation also occurs during tissue inflammation as a result of cellular enzymatic release (Davis, 1992). The resulting denatured protein, known as gelatin, contains previously masked RGD (Arg-Gly-Asp) sequences that allow for cell adhesion using RGD binding receptors (Agrez et al., 1991; Davis, 1992). This alteration in the normal profile of adhesion site availability may alter the biologic properties of the protein and act as a signaling cue for cells (Jha et al., 2011). Gelatin has recently been used with HA in an attempt to mimic natural bone; scaffolds composed of gelatin, HA and gellan (added to improve the poor mechanical properties of gelatin) showed similarity to natural bone (in terms of chemical composition, homogeneity, molecular interactions and structural conformation) and supported adhesion and proliferation of human mesenchymal stem cells (Barbani et al., 2011).

Due to its abundance in numerous tissues and its multifunctional characteristics, collagens have been used to investigate a wide variety of cellular behaviors. The major functional cell adhesion peptide sequence that has been identified in collagen is the triple-helical GFOGER (Gly-Phe-O-Gly-Glu-Arg) sequence found in many collagens. The following integrins are known to bind to collagen ligands: $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ (Barczyk, 2010). Notably, due to their ability to bind cells, collagens can be used to investigate cell adhesion formation and migration in both 2- and 3-D. Additionally, collagen research has led to significant insights into the migration patterns of cell types that utilize collagenases as a means of remodeling a collagen rich matrix for efficient 3-D migration. Commercially available PureCol (previously Vitrogen) is a collagen solution that can form a network of collagen fibrils as either a thin layer or solid gel and provides an excellent environment for studying cell-protein interactions. Significant research using PureCol has been conducted on a variety of cell types and cell functions

Table 1 Major components of the native ECM that are commonly used for studying cell-ECM interactions. Adapted from Sell et al. (2010)

Major proteins of the native ECM			
Protein	Physiologic location	Function	Major cell adhesion peptide sequence
Collagen	Widely distributed	Structure Cell-matrix interactions	*GFOGER* (Gly-Phe-O-Gly-Glu-Arg)
Elastin	Elastic tissues (arteries, veins, ligaments, lung, skin, intestines, bladder)	Structure Elasticity	*VGVAPG* (Val-Gly-Val-Ala-Pro-Gly)
Fibronectin	Widely distributed	Blood clotting Cell-matrix interactions Matrix-matrixinteractions	*RGDS* (Arg-Gly-Asp-Ser) *LDV* (Leu-Asp-Val)
Laminin	Basal lamina	Structure Cell-matrixinteractions	*IKVAV* (Ile-Lys-Val-Ala-Val) *YIGSR* (Tyr-Ile-Gly-Ser-Arg)

including cancer cell invasion and proliferation, cardiac fibroblast remodeling and transplanted fibroblast survival in spinal cord injury (Werbowetski et al., 2004; Hayashi et al., 2005; Paik et al., 2006). Lyophilized collagen and gelatin is also commercially available and has been highly utilized in scaffold fabrication using the process of electrospinning (Matthews et al., 2002; Ayres et al., 2006, 2007). Collagen-based electrospun scaffolds have been investigated for their functionality in mediating cell infiltration and migration *in vivo*, for use as wound healing templates and for vascular engineering applications (Telemeco et al., 2005; Ju et al., 2010; Jha et al., 2011).

Elastin

Elastin is a critical structural component that imparts elastic recoil properties and resilience in tissues that are subject to repetitive distension and stress. This protein constitutes the walls of arteries and veins, ligaments, lung, skin, intestines and bladder (Table 1) (Rodgers and Weiss, 2005). Tropoelastin, the precursor to elastin, is a soluble and hydrophobic protein and when covalently cross-linked forms elastin, which is chemically inert and insoluble (Sell et al., 2010). Elastin has been shown to modulate cellular physiology, effecting signaling, chemotaxis, proliferation and protease release of a variety of cell types including fibroblasts, smooth muscle cells, endothelial cells, chondrocytes, monocytes, macrophages, neutrophils and lymphocytes (Duca et al., 2004). The cellular elastin binding protein (EBP), a multi-functional membrane protein, recognizes the peptide sequence VGVAPG (Val-Gly-Val-Ala-Pro-Gly) and appears to have a potent effect on cellular responses; when coated onto plastic dishes or the lower portion of a Boyden chamber, this elastin-derived peptide supported increased proliferation and chemotaxis of monocytes and fibroblasts (Rodgers and Weiss, 2005). Additionally, cells have exhibited adhesion to elastin via integrin $\alpha_v\beta_3$, elastonectin (a protein with a molecular weight that closely corresponds to that of an integrin subunit) and G protein-coupled receptors (Rodgers and Weiss, 2005). In investigations of ECM protein cellular signaling, tropoelastin (the precursor to elastin) has been examined as a possible coating for intravascular devices; stainless steel disks coated with tropoelastin promoted endothelial cell adhesion, migration and the development of a monolayer able to withstand and respond to arterial shear stress (Wilson et al., 2011).

Fibronectin

Fibronectin is a glycoprotein with multiple domains that can bind simultaneously to cell adhesion molecules, ECM proteins (such as collagen, heparin and fibrin), glycosaminoglycans (GAGs) and other fibronectin molecules (Schwarbauer and DeSimone, 2011). This protein is widely distributed in the body and plays a vital role in a number of cell processes (Table 1). One clear physiologic example of fibronectin-mediated cellular activity is fibroblast and neutrophil migration into a wound bed. Despite their differing functions (neutrophils phagocytose debris and bacteria while fibroblasts are responsible for laying down the collagen matrix that becomes new tissue) it appears that fibronectin is important for guiding their migratory activity by promoting adhesion, motility and chemotaxis for both of these cell types (Grinnell, 1984). Fibronectin consists of two nearly identical polypeptide chains, appears as a rod-like structure and exists in a variety of isoforms generated by alternative RNA splicing (Singh et al., 2010; Schwarbauer and DeSimone, 2011).

Both a soluble plasma and insoluble cellular type of fibronectin exist; plasma fibronectin circulates in the blood and is critical for blood clotting, while cellular fibronectin is present in most tissues and has a variety of roles ranging from joining neighboring cells to facilitating collagen fibril accumulation (Singh et al., 2010). Insoluble cellular fibronectin is formed by soluble fibronectin typically secreted by fibroblasts; this complex process involves the binding of soluble fibronectin to cell surface integrins (typically $\alpha_4\beta_1$), leading to integrin clustering and fibronectin interactions between local molecules. Short fibronectin fibrils then form between adjacent cells. Fibronectin adherent cell stretching causes the fibronectin molecule to partially unfold, exposing binding sites that promote fibronectin-fibronectin interactions and lead to the formation of an insoluble ECM (Wierzbicka-Patynowski and Schwarzbaurer, 2003). Fibronectin appears to often function in cooperation with collagen; collagen and fibronectin are commonly located together and fibroblasts secretory pathways contain colocalized collagen and fibronectin (Ledger et al., 1980). Researchers have also shown that collagen fibrils do not accumulate in the body in the absence of fibronectin and it is hypothesized that fibronectin matrix orientation aids in aligning collagen fibrils (Singh et al., 2010). Additionally, it appears that fibronectin (when bound to lysyl oxidase or LOX, the enzyme that facilitates covalent cross-linking in elastin fibers) may aid in the rearrangement of tropoelastin into elastin fibers (Wagenseil and Mecham, 2007).

The following integrins are known to bind to fibronectin ligands: $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_{IIB}\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_3$,$\alpha_v\beta_6$ and $\alpha_4\beta_7$ (Barczyk et al., 2010). The major identified functional cell adhesion peptide sequences in fibronectin are RGDS (Arg-Gly-Asp-Ser) and LDV (Leu-Asp-Val); LDV is present in the alternatively spliced region of the protein (Barczyk et al., 2010). Investigations of fibronectin mediated cellular activity have primarily centered around the fibronectin connecting segment-1 (CS-1) peptide sequence, which contains the LDV sequence and whose receptor is the integrin $\alpha_4\beta_1$. This sequence was originally identified by its ability to promote melanoma cell adhesion and has been shown to support leukocyte adhesion to endothelial cells and monocyte rolling and adhesion to atherosclerotic lesions (Komoriya et al., 1991; Huo et al., 2000; Man et al., 2009). Additionally,

interactions between integrin $\alpha_4\beta_1$ and the CS-1 peptide appear to support not only cell rolling and adhesion but motility as well (Wu et al., 1995).

Laminin

Laminin, a major structural component of the ECM, is composed of three polypeptide chains (α, β and γ) bound through disulfide bonds. These cross-shaped trimers exhibit at least 15 different isoforms (many of which are not functionally understood) and have tissue and development-specific distributions. Laminin is widely distributed throughout the body and is present in the basil lamina of basement membranes in essentially all tissues (Table 1). Each laminin trimer contains a large globular domain at the base of the cross-shaped molecule; this large globular domain represents the primary site for the interactions of laminin with cell surface receptors (Sugawara et al., 2008). The other three laminin "arms" are excellent at binding to other laminin molecules, making this protein excellent at forming sheets.

Similar to collagen, laminin functions as both a structural (mediating cell-cell connections and ECM assembly) and biologic (supporting cell adhesion, migration and differentiation) component of tissue. Laminin plays a critical role in muscle and nerve maintenance and development; deficiency in the structural development of the α2 chain of laminin appears to cause a number of congenital muscular dystrophies that are also associated with peripheral and central nervous system neurological defects (Colognato and Yurchenco, 2000; Guo et al., 2003; Tzu and Marinkovich, 2008). Laminin-332 is necessary in the skin, placenta and mammary glands for hemidesmosome formation and cell migration while laminin-411 is vital for neutrophil migration and extravasation (Tzu and Marinkovich, 2008). Additionally, a number of functional laminin cell adhesion peptide sequences have been identified and studied; IKVAV (Ile-Lys-Val-Ala-Val) has been demonstrated to promote endothelial cell migration and angiogenesis while YIGSR (Tyr-Ile-Gly-Ser-Arg) appears to block endothelial cell differentiation into capillary structures (Simon-Assmann et al., 2011).The following integrins are known to bind to laminin ligands: $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_7\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (Tzu and Marinkovich, 2008; Barczyk et al., 2010). Absent or defective laminin receptors can lead to a variety of conditions; for example, defective $\alpha_6\beta_4$ integrins can lead to a painful skin blistering disorder (known as junctional epidermolysis bullosa with pyloric atresia) while absent $\alpha_7\beta_1$ integrins (on monocytes) have been observed in congenital muscular dystrophy (Tzu and Marinkovich, 2008).

Matrigel

Matrigel is a gelatinous protein mixture extracted from Engelbreth-Holm-Swarm tumors in mice sarcoma cells. This mixture is composed primarily of laminin-111, collagen IV, fibronectin, entactin/nidogen-1 and a variety of growth factors (such as fibroblast growth factor, transforming growth factor beta, epidermal growth factor, platelet derived growth factor and insulin-like growth factor) and mimics the complex ECM found throughout the body (Hughes et al., 2010; Benton et al., 2011). The array of proteins present in Matrigel allows for the presentation of a variety of native adhesive peptide sequences to cells and subsequently stimulates complex cellular processes. However, while Matrigel can be produced with growth factor enriched or depleted versions of the protein mixture, complete control over the final protein composition is difficult and can vary between lots. Because of its complex composition, Matrigel can be applied to investigations of multiple cell types and tissue specific cell responses. McCracken et al. were able to generate 3D human intestinal tissue from human pluripotent stem cells (hPSCs); using a series of differentiating and patterning steps they were able to produce 3D spheroids which, following long-term culture on Matrigel with prointestinal growth factors, gave rise to intestinal tissue (McCracken et al., 2011). Additionally, neonatal rat cells cultured in a collagen/Matrigel scaffold (fabricated using a casting mold) self-assembled into renal-like tissue and exhibited tubules and glomeruli like structures (Lü et al., 2011).

While there are abundant benefits to the use of natural proteins for investigation of cell adhesion molecule mediated functions, there also exist inherent problems with their use. These would include degradation issues, poor mechanical properties and inconsistencies during processing due to temperature and pH sensitivity, ultimately leading to lot-to-lot variability (Barnes et al., 2007). Additionally, as these proteins are naturally found in the body, many cell types will respond to the multiple bioactive domains found within any single protein. The complexity related to presentation of multiple bioactive domains leads to difficulty in isolating individual adhesion molecule mediated cellular response. These issues, inherent to the use of natural proteins, can be avoided through the use of artificially engineered extracellular matrix mimetics.

Artificial extracellular matrix scaffolds

Artificial ECM scaffolds are valuable tools for evaluating cell-ECM interactions that modulate cell function. These scaffolds can be composed of a variety of polymers, natural or synthetic in origin (or a combination of both) and may be modified to include bioactive peptide sequences, growth factors, proteases, or cytokines. The matrix fabrication methods reviewed below are only a small representation of the many successfully utilized scaffold production techniques.

Composition

Artificial matrix scaffolds are fabricated using polymers, long chain molecules composed of repeating structural units, which may be synthetic or natural in origin. Synthetic polymers commonly used in matrix engineering are polyglycolic acid (PGA), polylactic acid (PLA), polydioxanone (PDO), polycaprolactone (PCL), polyethylene glycol (PEG), used alone or as blends and/or hybrids. Recent tissue engineered vascular graft advancements by the Breuer group have involved the use of biodegradable scaffolds composed of a synthetic PGA mesh and a copolymer sealant solution of PLA and PCL (Roh et al., 2010). These synthetic scaffolds were seeded with bone marrow mononuclear cells prior to implantation into the mouse inferior vena cava (IVC). After 24 weeks the biodegradable scaffold had transformed into a functional blood vessel, resembling native mouse IVC with layers of endothelial cells, smooth muscle cells and collagen fibrils (Roh et al., 2010). Additionally, biodegradable poly-L-lactic acid (PLLA) was used with the natural bone mineral HA to produce scaffolds for bone tissue engineering; these scaffolds successfully supported osteoblast adhesion, differentiation and survival (Ma et al., 2001; Woo et al., 2007). Scaffolds composed of synthetic materials are often utilized in this manner; the excellent biodegradability profile of certain synthetic polymers allows them to provide initial tissue structure prior to native cells protein deposition and ECM development.

Aside from their biodegradability, another advantage of synthetic polymers is their excellent mechanical properties; these properties can often only be achieved in natural polymers by scaffold fixation/cross-linking. Recent research has focused on utilizing a combination of both synthetic and natural polymers for tissue engineering scaffolds in an attempt to overcome the issues of each class of polymer. For example, McClure et al. fabricated multi-layered electrospun scaffolds composed of PCL, elastin and collagen in an attempt to mimic the mechanical properties of native arterial tissue (using the synthetic polymer) while encouraging native tissue regeneration (using the natural polymers) (McClure et al., 2010).

Additionally, the natural polymers typically used in matrix engineering are those widely distributed throughout the body (collagen, elastin, fibronectin, laminin, etc.). These natural polymers contain cell adhesion sites that are necessary for directing cell processes in healthy and diseased tissue. Natural proteins are composed of amino acid sequences, or peptide sequences; some of these regions within the proteins are bioactive and interactions between cell surface receptors and these peptide sequences direct a variety of cellular processes. Many of these bioactive peptide sequences have been delineated for a number of proteins and cell surface receptors and synthetic versions are easily produced. These peptide sequences are typically polymerized to other polymers in order to functionalize or manipulate synthetic or natural materials (Gonzalez et al., 2004, 2006).

Fabrication methods

Cell-ECM interactions may be researched using two-dimensional surfaces; while these assays provide significant information, three-dimensional matrices more closely approximate and mimic cell behavior and functionality in native tissue. These matrices can be produced with a variety of synthetic polymers and natural proteins and are easily modified with the addition of cell adhesion peptides, cytokines and growth factors (Saltzman et al., 1992). A number of techniques to fabricate matrices have been developed and range from simple protein gels to complex self-assembled matrices; below we will briefly review a few of these matrix fabrication methods.

Hydrogels are hydrophilic cross-linked polymeric membranes and may be classified as homopolymer, copolymer or multipolymer (Fig. 2) (Gonzalez et al., 2004). Homopolymer

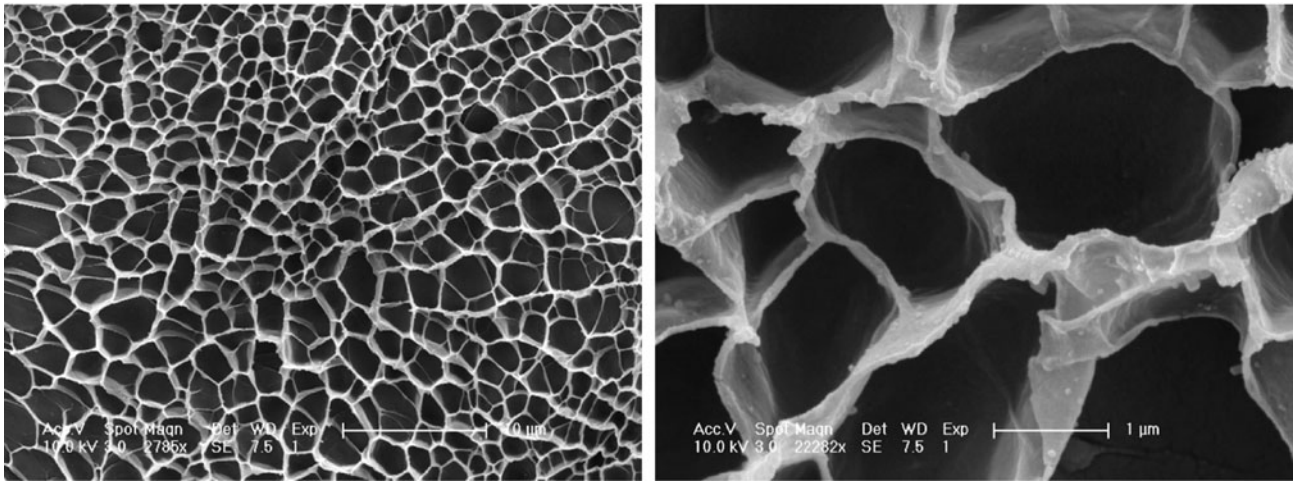

Figure 2 SEM images of a PEG hydrogel. PEG hydrogels were freeze-dried and gold sputter coated in preparation for SEM imaging. Left: approximately 2800×magnification, Right: approximately 22000×magnification.

hydrogels are composed of one type of monomer, copolymer hydrogels of two types of comonomer units and multipolymer hydrogels of three types of comonomer units (Peppas, 2004). Copolymer and multipolymer hydrogels must contain at least one hydrophilic comonomer to allow for swelling and to resist protein adsorption (Gonzalez et al., 2004). Hydrogels are prepared by chemically cross-linking, photopolymerizing, or irradiative cross-linking of a polymer and the hydrogels are then swelled in water or phosphate buffered saline (PBS) (Peppas, 2004). A wide variety of natural and synthetic polymers can be used to produce hydrogels and structural and mechanical modifications are easily made by altering the molecular weight of the polymer and the cross-linking density within the hydrogel (Lee and Mooney, 2001). Hydrogels can also be modified to incorporate synthetic specific peptide sequences (1) found in natural proteins to support and influence cellular activities and (2) sensitive to degradation by enzymes (such as collagenase) and matrix metalloproteinases (MMPs) to create a three-dimensional matrix to allow for cell migration (Gobin and West, 2002; Lee et al., 2007; Andukuri et al., 2010). The major advantage of hydrogels is the variety of polymers and ability to incorporate peptide sequences; however, issues do exist regarding mechanical properties and reproducibility.

Electrospun scaffolds are produced by using an electric field to process a polymer solution into a fibrous construct. A polymer solution is prepared and deposited into a syringe with a blunt tipped needle, charged to a high potential and directed toward a grounded target mandrel. The polymer solution is then fed at a metered rate into the electric field; as the electric potential drives the solution across the air gap the carrier solvent evaporates and discrete fibers are deposited as a non-woven matrix. The process of electrospinning allows for the production of very small diameter fibers, interconnected pores and material and biologic properties that can be regulated at several sites in the electrospinning process. Physical properties, including fiber diameter and pore dimension, can be regulated by controlling the composition of the electrospinning solvent and the identity, concentration, and/or degree of chain entanglements (viscosity) present in the starting polymer(s) (Fig. 3) (Matthews et al., 2002). Fiber alignment and overall scaffold anisotropy can be selectively controlled by mandrel speed and polymer concentration; higher mandrel speeds and polymer concentrations yield more highly aligned scaffolds (Ayres et al., 2006, 2007). Overall, electrospinning is simple, inexpensive and versatile, allowing for a wide range of production materials and scaffold structure. However, the disadvantages of electrospinning are the production of fibers that are in the upper range of those found in native tissues and the use of organic solvents.

The phase separation technique has long been used to produce porous polymer membranes and has been refined more recently by Ma and Zhang to produce nano-fibrous matrices (van de Witte et al., 1996; Ma and Zhang, 1999). There are four main techniques utilized when producing matrices using this process: thermally induced phase separation, air-casting of a polymer solution, precipitation from the vapor phase and immersion precipitation (van de Witte et al., 1996). The most common method, thermally induced phase separation (TIPS), begins with dissolving a polymer in a solution (typically tetrahydrofuran/THF) and phase separating them thermally, creating a gel (Ma et al., 2005). Next, the solvent is extracted from the gel and the gel is frozen and freeze-dried under vacuum (Vasita and Katti, 2006). In this technique, polymer gelation time and temperature controls porosity and pore size and structure can be modified by a number of porogens, such as sugar, inorganic salt and paraffin spheres (Zhang and Ma, 2000; Chen and Ma, 2004; Barnes et al., 2007). This fabrication technique is simple and produces consistent results; however, issues regarding porogen extraction and the small number of usable polymers limit the practical use of this method.

Self-assembly is the organization of disordered molecules into structures or patterns without external intervention. Self-assembled matrix fabrication is limited to only a few polymer configurations (diblock copolymers, triblock copolymers, triblocks from peptide-amphiphile and dendrimers) (Jayaraman et al., 2004). The most commonly researched self-assembled molecules are peptide amphiphiles (PAs); these peptide-based molecules were initially developed by Berndt et al. to examine the interaction between peptide structures and ligands (Berndt et al., 1995). PAs are composed of a hydrophilic peptide head group (a N-alpha amino group) attached to a hydrophobic tail group (a dialkyl chain moiety). In water, the hydrophobic alkyl tail groups cluster together,

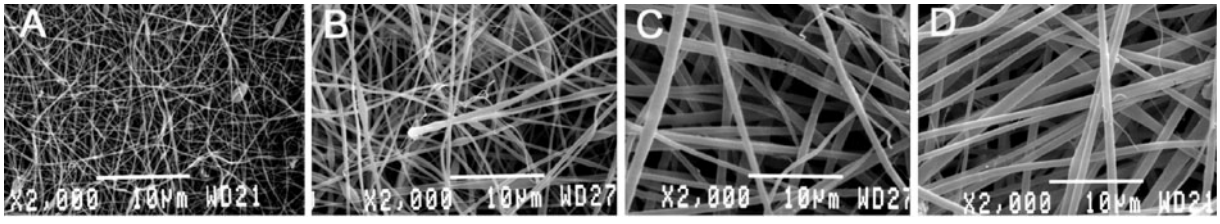

Figure 3 SEM images of electrospun collagen. Fiber diameter can be controlled by the initial electrospinning starting concentration; increased starting concentrations result in increased fiber diameter. Type I collagen was solubilized in 2,2,2-Trifluoroethanol and electrospun at starting concentrations of (A) 40 mg/mL, (B) 60 mg/mL, (C) 80 mg/mL and (D) 100 mg/mL. All images are 2000×magnification.

leaving the hydrophilic head group exposed (Ma et al., 2005; Vasita and Katti, 2006). The PAs are then treated with acid (typically a dithiothreitol solution) and the pH is rapidly reduced to create insoluble fibers (Barnes et al., 2007). Additionally, this method of matrix fabrication can also be applied to hydrogel production by using iso-electric focusing to drive collagen fibers to self-assemble (Abu-Rub et al., 2011). The major advantage of this fabrication technique is that it can be used to produce fiber sizes that closely approximate those of the native ECM; however, the high complexity of this method and issues with mechanical properties limit the use of this technique.

Methods of analysis

Cell function as influenced by cell-ECM interactions is primarily analyzed using microscopic techniques, ranging from simple phase-contrast microscopy to more complex confocal microscopy. Cell adhesion, spreading, motility, and phagocytosis can easily be analyzed using time-lapse video or digital microscopy. Digitally imaging a field of cells before and after adhesive competition or inversion assays is an effective and simple way to calculate cell adhesion percentages. Individual cell spreading and motility may be analyzed by quantifying changes in cell surface area, polarization, distance from origin and cell velocity using time lapse video microscopy (Gonzalez et al., 2004, 2006). A quantification of cell shape change and motility may be conducted using microscopic images and the Fourier transform (Partin et al., 1989). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are methods by which cell morphologic observations are made; however, the processes used to prepare a sample for SEM or TEM may corrupt the original sample (Wang et al., 2010). The "wound scratch assay" is a popular model for examining cell migration; a wound is created on a two-dimensional surface by scratching a needle across a confluent monolayer of cells and time lapse video microscopy is used to analyze wound closure (Friedl, 2004). Phagocytic examination is possible using video time-lapse microscopy and zymosan particles to initiate phagocytosis (Macrae and Pryzwansky, 1984; Underhill, 2003). Additionally, microscopic evaluation of targeted fluorescent microbeads has been used to quantify phagocytic activity (Handley et al., 2005).

Sample fixation, staining and immunofluorescence imaging (using light or confocal microscopy) is a method used to examine stress fiber formation and adhesion molecule expression (Maheshwari et al., 2000; Andukuri et al., 2010; Barnhart et al., 2011; Jha et al., 2011). Methods to analyze cell differentiation and apoptosis typically utilize techniques such as immunoprecipitation, western blotting, and flow cytometry (Pluskota et al., 2008; Chen et al., 2012).

Adhesion

Cell adhesion, the act of cell binding to an ECM or other cell, is a necessary precursor to basic cellular processes (such as cell proliferation, motility, differentiation, apoptosis and tissue development) and is mediated by cell adhesion molecules such as integrins and selectins. A major recognition system in cell adhesion, the three amino acid sequence RGD, was first discovered in fibronectin and has since become recognized as a cell attachment site for many adhesive proteins (Pierschbacher and Ruoslahti, 1984). The RGD adhesion sequence is now recognized as the prototype adhesion signal in cell adhesion biology and due to its small structure can be easily reproduced synthetically with peptides. When coated on a surface the RGD peptide promotes cell adhesion and when added to a cell solution the RGD peptide occupies receptors and blocks cell adhesion. At least 8 (and as many as 12) integrins recognize the RGD adhesion sequence in their ligands ($\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_{IIb}\beta_3$). The following is a partial list of adhesion proteins containing RGD sites: fibronectin, vitronectin, fibrinogen, thrombospondin, laminin, entactin/nidogen, tenascin and under some conditions, collagen (Ruoslahti, 1996). Large fibronectin fragments preferentially bind to the $\alpha_5\beta_1$ integrin (known as the fibronectin receptor) while smaller fragments bind better to the $\alpha_v\beta_3$ integrin (known as the vitronectin receptor); it appears that while integrin clustering of $\alpha_5\beta_1$ is responsible for maintaining adhesion strength, $\alpha_v\beta_3$ initiates mechanotransduction and reinforces integrin-cytoskeleton links (Roca-Cusachs et al., 2009).

The RGD sequence may not always be available for cell adhesion at the surface of a protein or may not have sequence homology that allows for cell adhesion; cell binding specificity can be achieved by placing specific residues around the RGD sequence of the peptide. For example, the GRGDSP hexapeptide is 1000 times less active than fibronectin and is fairly specific in its binding activity; however, replacing the aspartic acid residue with a glutamic acid residue increases the specificity of the peptide sequence 100-fold. Additionally, the conformation of the amino acids is critical; variances in the form of the amino acid can activate or inactivate a peptide sequence (Ruoslahti, 1996).

RGD peptide sequences have provided significant insight into cellular adhesion mechanisms. Gonzalez et al. utilized immobilized RGDS (Arg-Gly-Asp-Ser) and TMKIIPFNRL-TIGG (Thr-Met-Lys-Ile-Ile-Pro-Phe-Asn-Arg-Ser) peptide sequences in PEG hydrogels as an investigational tool for understanding human neutrophil adhesion and spreading. Assays were conducted using Smith-Hollers chambers and demonstrated that neutrophils adhered to RGDS-PEG hydrogels (even without stimulation) and adhesion improved with higher concentrations of the RGDS peptide sequence in the PEG hydrogel. When stimulated, neutrophil spreading on

RGDS-PEG hydrogels was significantly increased. Blocking assays indicated that neutrophil adhesion to RGDS appears to involve both $\alpha_v\beta_3$ and β_1 integrins while neutrophil spreading involves only the β_1 integrin. Neutrophils seeded onto hydrogels modified with both RGDS and TMKIIPFNRL-TIGG demonstrated a dramatic increase in cell spreading, suggesting that adhesive interactions between peptide sequences result in cell shape changes that precede cell migration (Gonzalez et al., 2004). Additionally, a number of studies involving the influence of the RGD peptide sequence on functional cell adhesion and spreading have been successfully conducted with other matrix fabrication methods; for example, Wang et al. investigated the compatibility of NIH 3T3 cells with RGD-modified electrospun chitosan nanofibers and demonstrated increased cell adhesion and proliferation (Wang et al., 2010).

It is also possible to modulate cell adhesion interactions through controlled degradation of natural proteins by thermal denaturation or hydrolysis. Jha et al. were able modify fibroblast functionality and adhesion by a stepwise thermal denaturation of type I collagen prior to electrospun scaffold fabrication. Dermal fibroblast adhesion to electrospun scaffolds composed of denatured collagen was increased until a threshold was reached at collagen thermally denatured at 70C; most likely, this scaffold was composed of a mixture of intact and destroyed triple helix and an optimal number of cryptic RGD binding sites were exposed, mediating strong adhesive interactions (Jha et al., 2011).

The use of micropatterning, microposts, microstencils, micropillars and nanogratings are recently developed methods of studying cell response. These topographical alterations can be used to obtain a variety of measurements such as basic cell response (adhesion, motility, proliferation, alignment and elongation), cellular traction forces, focal adhesion recruitment and intercellular adhesion forces (Tan and Saltzman, 2002; Sniadecki et al., 2007; Ghibaudo et al., 2009; Koo et al., 2011; Long et al., 2011; Kafi et al., 2012). Microfabrication technology has made it possible for researchers to examine topographical effects on cell behavior and these results reveal invaluable information on how matrix topography can influence cell behavior and processes. Kafi et al. (2012) developed three-dimensional RGD peptide nanostructured arrays using the mask-assisted self-assembly technique. 3D-RGD nanostructures were fabricated at varying heights on gold (Au) surfaces and assays examining cell spreading, adhesion and proliferation revealed that these cell processes were improved on 3D-RGD nanostructures when compared to 2D-RGD monolayers or 2D-RGD nanodots.

While a significant number of integrins recognize RGD in their adhesion protein ligands, a number of other peptide sequences exist that also support cell adhesion. Some peptide sequences are slight variations of the RGD sequence; for example, the KGD sequence can bind to the $\alpha_{IIb}\beta_3$ integrin and the RHD sequence can bind to the $\alpha_5\beta_1$ integrin (Ruoslahti, 1996; Matter et al., 1998). Additionally, the peptide sequence found in collagens, GFOGER, is bound to by integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ (Barczyk et al., 2010).

In vascular applications, it may be important to utilize proteins and/or peptide sequences that do not contain bioactive RGD sequences as they promote the attachment of platelets, which can lead to thrombosis. Andukuri et al. (2010) demonstrated the effects of the YIGSR (found in laminin, bound by cells with the laminin binding region of the β_1 integrin) and VAPG (Val-Ala-Pro-Gly) (a non-integrin binding elastin ligand) peptide sequences on human umbilical vein endothelial cells (HUVECs) and aortic smooth muscle cells (AoSMCs). Nanomatrices were developed with self-assembled PAs using a solvent evaporation technique. These matrices also contained a MMP-2 degradable peptide sequence, GTAGLICQ (Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln), to allow for matrix remodeling. Cell adhesion was evaluated with a fluorometric DNA assay, cell spreading with a live/dead assay and stress fiber formation with a F-actin rhodamine-phalloidin staining. Results demonstrated that PA-YIGSR enhanced HUVEC adhesion and spread morphology and stress fiber formation. PA-YIGSR and PA-VAPG improved AoSMC adhesion and PA-VAPG enhanced AoSMC spreading. Both PA-YIGSR and PA-VAPG had reduced platelet adhesion compared with the collagen type I control.

Cell adhesion may also be mediated by materials that are not human in origin and do not contain the typical adhesion sequences. For example, chitosan is a common component of scaffolds fabricated for bone tissue engineering and successfully supports cell attachment and proliferation; this functionality is attributed to the structurally similar polysaccharide backbone of chitosan compared to glycosaminoglycan (a major component of bone ECM) (Thein-Han and Misra, 2009). Chitosan-HA composite scaffolds cultured with pre-osteoblasts exhibited excellent levels of cell attachment and proliferation over 7 days (Thein-Han and Misra, 2009). Chitosan-polyethylene oxide (PEO) nanofibers also exhibited excellent osteoblast and chondrocyte adhesion (Bhattarai et al., 2005).

Motility

Although cell migration is commonly considered the movement of individual cells (which includes polarized extension and contraction cycles with adhesion and de-adhesion to the surrounding matrix substrate), this term also includes the process of collective cell migration, or the movement of a group, sheet or strand of mobile cells that are connected by cell-cell junctions. During single cell migration, a cyclic 5 step process has been identified: (Step 1) Intracellular actin polymerization to cystoplasmic filaments leads to cell polarization and the extension of a leading pseudopod. (Step 2) Protruding cell extensions make contact with the

ECM and β_1 and β_3 integrins cluster and attach to the ECM and engage focal adhesions. (Step 3) Pericellular matrix breakdown provides room for the expanding cell body to penetrate into the ECM. (Step 4) Following integrin-ligand binding, actin filaments engage with cross-linking and contractile proteins in order to stabilize and contract. (Step 5) Actin contraction leads to retraction and forward gliding of the *posterior* cell pole and movement of the cell body including the nucleus to the leading edge (Friedl, 2004). For collective migration, cells maintain cell-cell junctions; the leading edge of the cell group (which may be one or many cells) follows steps 1–4 of the single cell migration process. During step 5, the functions of leading edge extension and rear retraction are shared among coupled cells and other cells become passively dragged with the collective cell group. It appears that individual and collective migration states are temporary and that it is possible for cells to toggle between these two states depending on internal (molecular elements) and external (environmental) factors (Friedl et al., 2004).

The three-dimensional architecture of the ECM appears to provide information to cells that is not available when cells adhere to a two-dimensional ligand coated surface. During attachment to three-dimensional collagen matrices, many cells upregulate $\alpha_2\beta_1$ expression; the same phenomenon is not seen on cell adhesion to two-dimensional collagen surfaces (Fig. 4) (Friedl and Bröcker, 2000). Meshel et al. (2005) demonstrated that in three-dimensional collagen matrices non-muscle myosin heavy chain II-B (NMHC II-B) deficient fibroblasts demonstrated lower rates of gel contraction and cell movement. Conversely, NMHC II-B deficient fibroblasts on two-dimensional collagen surfaces exhibited no change in collagen bead and cell movement.

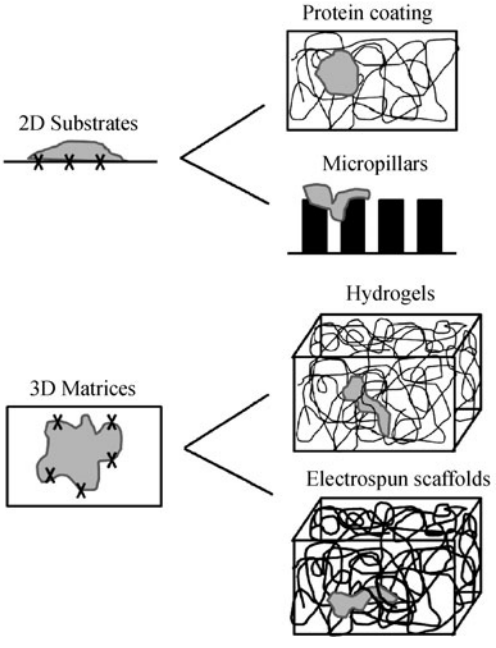

Figure 4 2D surfaces vs 3D matrices. 3D matrices more closely approximate the native ECM environment and induce cell reactions and responses that mimic those *in vivo*. Cells cultured on 3D matrices exhibit increased cell movement and integrin expression when compared to cells cultured on 2D surfaces. Labeling: 'x' on the cells denotes cell adhesion molecules.

Cell migration into tissue involves binding interactions between cell-surface receptors and ECM ligands, which provides the mechanical traction needed to propel a cell forward and generate signals that regulate cell behavior. To examine this cell-substrate mediated motility in three-dimensions, Parkhurst and Saltzman (1992) encapsulated neutrophils in type I collagen gels and tracked individual cells. The results indicated that mean displacement and motility of a neutrophil is dependent on the collagen concentration of the surrounding gel. This phenomenon is based in part on the adhesive strength of the surface (and may also be a function of the changing viscosity and flexibility of the collagen gel); if cell-surface adhesion strength is low, cells cannot gain traction to migrate forward and if cell-surface adhesion strength is high, cells become fixed to the surface and will not migrate. Below a collagen gel concentration of 0.1 mg/mL cells were not able to adhere sufficiently and subsequently sank through the gel; at collagen concentrations above 0.7 mg/mL cells were immobilized in the gel. Maximal motility was exhibited at a collagen concentration of 0.3 mg/mL indicating that inter-collagen fiber spacing and cell-fiber interactions appear to be optimal at this concentration.

Maheshwari et al. (2000) also demonstrated the critical role of cell-ECM interactions by examining NR6 fibroblast motility in inert polyethylene oxide (PEO) hydrogels containing epidermal growth factor (EGF) and varying surface density and spatial distribution of YGRGD (Tyr-Gly-Arg-Gly-Asp) peptide sequences. Their findings revealed that migration speed is a function of surface ligand density. Furthermore, increased ligand clustering reduced the ligand density necessary for cell migration. Non-clustered ligands supported cell adhesion but not cell migration. Their results indicate that cell migration can be regulated by varying ligand spatial distribution and that integrin clustering is necessary for cell motility.

While β_1 and β_3 integrins are regarded as the most important cell binding integrins, the membrane activated complex-1 (Mac-1) β_2 integrin also appears to play a role in mediating cell migration. Saltzman et al. (1999) showed that neutrophils in collagen gels of low concentration (with minimal ligand density) appear to depend on β_2 integrin expression for motility. Anti-CD18 (β_2 integrin Mac-1) antibodies decreased cell motility at low collagen concentrations but had no effect on cell motility at high collagen concentrations. Gonzalez et al. (2006) evaluated neutrophil motility on polyethylene glycol (PEG) hydrogels containing RGDS and TMKIIPFN-RTLIGG (P2, a Mac-1 ligand) peptide sequences alone and in combination. Neutrophil motility was not supported on RGD-PEG hydrogels but combined presentation of RGD and P2 on PEG hydrogels enhanced neutrophil motility. Naïve neutrophil motility on

combined substrate hydrogels was dependent on Mac-1 while integrins $\alpha_4\beta_1$ and $\alpha_6\beta_1$ influenced speed and linear movement. Transendothelial migrated neutrophil motility on combined substrate hydrogels was dependent on integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ while integrins $\alpha_4\beta_1$ and $\alpha_6\beta_1$ influenced speed and linear movement. These results suggest that crosstalk between Mac-1 and β_1 integrins provides intracellular signaling that is necessary for successful migration.

Phagocytosis

Phagocytes, including neutrophils, monocytes, macrophages and dendritic cells, are cells of the immune system that protect the body by ingesting harmful foreign or cellular debris, bacteria, parasites and dead (or dying) cells. Much of what is understood about innate immunity and the mechanisms of phagocytosis was discovered using a model microbial particle, zymosan, an insoluble cell wall preparation of *Saccharomyces cerevisiae* (Underhill, 2003). Monolayers of neutrophils incubated with zymosan particles exhibit a well-defined series of events that lead to phagocytosis. First, cell surface ridges and lamellipodia make contact with several zymosan particles before the lamellipodia form a cup around the zymosan and engulf the particle in a large cell cavity. Next, the zymosan particle is further surrounded by a forming phagosome (a vacuole formed by fusion between the cell membrane and zymosan particle) and eventually the lamellipodia edges meet, fuse and complete enclose and internalize the zymosan particle. Killing and digestion of the particle involves granular secretion into the phagosome and usually occurs before the particle is entirely ingested (Macrae and Pryzwansky, 1984).

Quantifying phagocytic activity may be difficult using standard methods; flow cytometric analysis is commonly used for fluid phase cells but there is no reliable method for measurement of phagocytic activity in adherent cells. Handley et al. developed the use of phycoerythrin (PE)-conjugated antibodies for use as target microbeads; these researchers used the targeted microbeads to examine the relationship between phagocytic capacity and morphology of monocyte derived dendritic cells. Following incubation with the targeted microbeads, incubation with FITC-conjugated antibody allows for the discrimination of internally versus externally bound beads and microscopic evaluation allows for phagocytosis quantification (Handley et al., 2005).

The role of the ECM on phagocytosis has long been researched and it is hypothesized that the ECM serves to signal phagocytic cells that have left the bloodstream and are in the extravascular tissue where maximum phagocytic activity is required. Brown examined the influence of monocyte, macrophage and neutrophil interactions with fibronectin and laminin and demonstrated enhanced phagocytic activity on all substrates (Brown, 1986). The collagen protein has also exhibited favorable properties with regards to improving phagocytic activity. Newman et al. demonstrated that monocytes phagocytized at a higher rate and 2.5 to 12-fold more bacteria when adherent to type I collagen gels compared to plastic. Additionally, monocytes exhibited similar phagocytic activity to 7 day cultured macrophages (Newman and Tucci, 1990). Fibrinogen appears to also enhance neutrophil phagocytic activity (Rubel et al., 2001).

To phagocytize harmful particles and debris, some cells must generate and release reactive oxygen species (ROS) in addition to granule contents. A number of substrate bound matrix proteins (fibrinogen, thrombospondin, laminin, fibronectin and vitronectin) initiate a respiratory burst in cytokine stimulated neutrophils. For neutrophils on fibrinogen, thrombospondin and laminin, this response appears to be Mac-1 integrin mediated while the integrin mechanism regarding fibronectin and vitronectin is unknown (Nathan et al., 1989). Tuluc et al. (2004) also demonstrated increased ROS production (during both N-formyl-methionine-leucine-phenylalanine (fMLP) or phorpbol 12-myristate 13-acetate (PMA) stimulation) along with enhanced primary granule release (during only fMLP stimulation) when neutrophils were incubated with soluble fibrinogen. These researchers demonstrated that fibrinogen modulation of neutrophil primary granule release is dependent on the Mac-1 integrin.

Differentiation

Cell differentiation and maturation are critical cell processes that specialize cell function during all stages of normal and diseased tissue homeostasis and development. Furthermore, differentiation and maturation are induced in part by cell signaling that arises from cell-ECM communications. Thus, cell adhesive interactions that occur with biomaterial ligands can induce certain cell function and behavior that can be manipulated to control cell responses to biomaterials.

Norton et al. examined dendritic cell (DC) maturation as a function of biomaterial substrate. Immature DCs were differentiated from human monocytes (using interleukin-4) and cultured on PLGA films; culture on PLGA films induced DC maturation while culture on agarose films did not (Norton et al., 2010). Mature DCs on PLGA films showed upregulation of β_2 integrins compared to agarose or tissue culture polystyrene (TCPS) surfaces (which did not induce DC maturation), indicating β_2 as a requirement for biomaterial induced DC maturation (Rogers and Babensee, 2011).

Naturally derived ECM can also act to guide cell differentiation; Chen et al. demonstrated the use of human amniotic membrane (containing a mixture of collagen, elastin, laminin and fibronectin) as a scaffold for the induction of osteogenic differentiation of mesenchymal stem cells for bone tissue engineering (Chen et al., 2012). Differentiation of osteogenic mesenchymal stem cells was also induced by culture on vitronectin and type I collagen; differentiation appeared to be substrate dependent as cells on vitronectin

demonstrated upregulation of focal adhesions while cells on type I collagen demonstrated downregulation of focal adhesions (Kundu and Putnam, 2006). Synthetic/natural composite scaffolds of PLLA and HA also promoted a highly differentiated osteoblast phenotype that expressed bone-specific markers (Ma et al., 2001). PC-12 nerve cell differentiation was induced more effectively on gelatin-PCL electrospun scaffolds compared to PCL electrospun scaffolds; further concluding that naturally derived protein signals act as biologic cues for cells and are necessary for the induction and control of cell processes (Alvarez-Perez et al., 2010). Along with the biologic and molecular characteristics of the ECM, surface topography can also influence cell differentiation. D1 cells, multipotent mouse bone marrow stromal precursors, appear to moderately differentiate into adipocytes when cultured on micropatterned poly PLLA substrates compared to plain PLLA or polystyrene (PS) (Chaubey et al., 2008). Béduer et al. (2012) were also able to control the differentiation of adult neural stem cells using micropatterned PDMS surfaces. Their results demonstrated that micropatterned surfaces can successfully be used as neurite guiding surfaces and the rate of cell differentiation can be controlled by channel width; cell differentiation was higher on micropatterned surfaces with a larger channel width. It appears that micropatterning (in terms of surface texture and channel width) may be a practical method for controlling the rate of cell differentiation.

Survival

Cell apoptosis, or programmed cell death, was originally believed to be controlled solely by hormones and growth factors but is now recognized to be mediated by ECM proteins. When deprived of adhesion to the ECM in the form of β_1 integrin antibodies, Meredith et al. revealed that, among its many other functions, the ECM acts a cell survival factor (Meredith et al., 1993). Cell apoptosis is critical for cell clearing from tissue but in some disease states cell death occurs prematurely or not at all, leading to issues including chronic inflammation and improper wound healing.

Neutrophils during the inflammatory response are a prime example of cell apoptosis that does not always take place at the precise time. It appears that the β_2 integrin and fibrinogen play a role in suppressing neutrophil apoptosis (Coxon et al., 1996; Rubel et al., 2001; Pluskota et al., 2008). Pluskota et al. (2008) examined neutrophil apoptosis as a function of the Mac-1 integrin ($\alpha_M\beta_2$ or CD11b/CD18). A number of Mac-1 ligands were examined; plasminogen, angiostatin (derived from plasminogen), fibrinogen and its two recognition peptides P1 and P2-C. Results demonstrated that plasminogen, fibrinogen and P2-C suppressed apoptosis while angiostatin and P1 did not prevent apoptosis. It appears that both the α_M and β_2 subunits must be engaged with pro-survival ligands in order to suppress apoptosis. These results infer that closely related ligands can have drastically different effects on cell survival and consequently, cell processes. Additionally, the same ligand can act in a cell-specific manner and have drastic effects on different cell types with regards to cell survival. The matrix protein CCN1 (CYR61) induces apoptosis in fibroblasts and promotes cell survival in endothelial cells. It appears that apoptosis in fibroblasts is controlled by its adhesion receptor $\alpha_6\beta_1$ while survival in endothelial cells depends on integrin $\alpha_v\beta_3$ (Todorovic et al., 2005).

Woo et al. (2007) used phase separation to fabricate PLLA/HA scaffolds for bone tissue engineering; osteoblasts cultured on these scaffolds demonstrated that PLLA/HA scaffolds promoted cell survival when compared to plain PLLA scaffolds. Further investigation demonstrated that the PLLA/HA scaffolds adsorbed significantly larger amounts of fibronectin, vitronectin, RGD and KRSR (an osteoblast adhesive peptide). β1 and β3 integrins and phosphorylation of focal adhesion kinase (FAK) and protein kinase B (PKB or Akt) proteins were upregulated on cells seeded onto PLLA/HA scaffolds with respect to plain PLLA scaffolds, inferring that this integrin-protein pathway protected the cells from apoptosis (Woo et al., 2007).

Conclusions

Interactions between cells and the ECM direct cellular processes in healthy and diseased tissue; this communication is mediated by a wide variety of adhesion molecules and ECM proteins (Fig. 5). These adhesion molecules currently undergo changes with regards to expression and conformation in order to appropriately moderate signals from the surrounding environment.While this review focused primarily on the contributions of integrins to outside-in signaling (these receptors have been widely studied) integrins work in conjunction with other adhesion molecules, such as cadherins, selectins and IgSF molecules, to guide cell processes. The ECM also undergoes constant remodeling by cells; these modifications contribute to the vital cell-ECM communication that guides cell function.

Bioactive regions within natural proteins can be extracted and presented as peptides in engineered artificial ECM. Natural or artificial matrices can be used to investigate cell activity, including adhesion, motility, phagocytosis, differentiation and survival that can be attributed to adhesion molecule interaction with distinct domains of matrix proteins. For many cell types, adhesion appears to be heavily dependent upon $\alpha_v\beta_3$ and β_1 integrins bound to protein peptide sequences RGD, VAPG and YIGSR (Andukuri et al., 2010; Wang et al., 2010; Jha et al., 2011). Transient tethering, or rolling, is supported by selectin heterotypic bonds (Varki, 1994; Golias et al., 2011). Motility appears to be dependent upon a number of integrin/matrix combinations, including Mac-1, $\alpha_v\beta_3$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ bound to collagen protein,

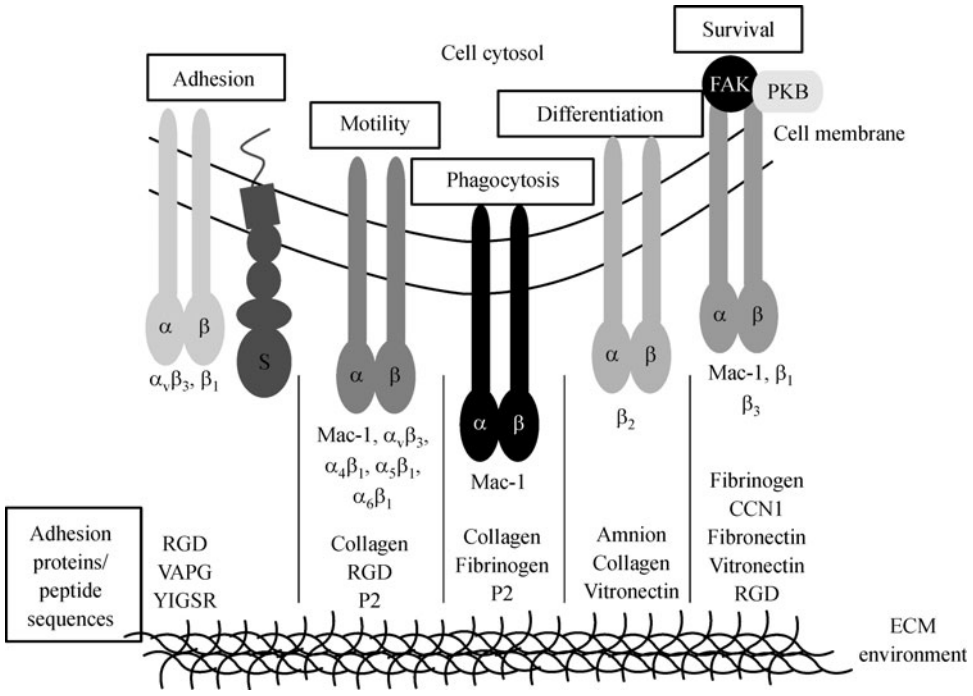

Figure 5 Schematic of cell function as mediated by specific cell adhesion molecules and adhesion protein/peptide sequences. Labeling: α and β denotes integrins, S denotes selectins, FAK denotes focal adhesion kinase and PKB denotes protein kinase B.

RGD, and P2 peptide sequences (Parkhurst and Saltzman, 1992; Saltzman et al., 1999; Maheshwari et al., 2000; Gonzalez et al., 2006). Phagocytosis has been associated with Mac-1integrin activity, and collagen, fibrinogen and the P2 peptide sequence have been shown to increase phagocytic activity (Brown, 1986; Nathan et al., 1989; Newman and Tucci, 1990; Rubel et al., 2001; Tuluc et al., 2004). Differentiation is β_1 dependent and amnion, collagen and vitronectin induced cell differentiation (Kundu and Putnam, 2006; Alvarez-Perez et al., 2010; Norton et al., 2010; Rogers and Babensee, 2011). Studies have indicated that survival is mediated by Mac-1, β_1 and $\beta_3$integrins, and binding to fibrinogen, fibronectin, vitronectin, and the RGD peptide sequence appear to suppress apoptosis (Meredith et al., 1993; Coxon et al., 1996; Rubel et al., 2001; Woo et al., 2007; Pluskota et al., 2008). Conversely, cell association with the ECM protein CCN1 seems to initiate apoptosis (Todorovic et al., 2005).

A wide variety of matrices can be successfully fabricated using a number of production methods. These matrices, including hydrogels, electrospun, self-assembled and phase separated can be composed of a variety of natural or synthetic polymers. Their structural and biologic properties can be tailored to the tissue under investigation. Additionally, topographically altered surfaces through micropatterning, microposts, microstencils, micropillars and nanogratings are also effective means of investigating adhesion molecule mediated cell function. Such engineered scaffolds allow for quantitative investigation of adhesion, motility, phagocytosis, differentiation and survival, as a function of cell adhesion molecule mediated interactions with the ECM. Examining how cell-ECM interactions modulate critical cellular processes is crucial to the relatively new field of tissue engineering. Evaluating ECM regulated cellular behaviors provides significant insight that will ultimately contribute to the future direction of this field: the development of a functional cell directing scaffold that can be used for organ replacement or tissue regeneration.

Bone engineering is an excellent example of a tissue engineering specialty that has successfully translated early research results, including how specific basic cellular activities can be induced by the ECM, into concepts that come together for the development of functional tissue repair and regeneration with an artificial ECM as the directing scaffold. Currently, a large number of bone graft options, including Osteograf, Norian SRS, ProOsteon, Osteoset, Cortoss, OPLA, Immis and OsteoScaf, are clinically available. Researchers in other areas are also making significant advancements toward the development of a functional tissue scaffold. For example, Roh et al. have developed a vascular graft composed of synthetic biodegradable materials (including PGA, PLA and PCL) that, following culture with bone marrow mononuclear cells and implantation into mice, transforms into a functional blood vessel (Roh et al., 2010). Additionally, the Niklason group has constructed lung scaffolds composed of decellularized rat lungs; when cultured with pulmonary epithelial cells, vascular endothelial cells and implanted into rats, these scaffolds demonstrated gas exchange for short time intervals (Petersen et al., 2010). These vascular grafts and lung scaffolds are just a few

examples of the most recent advancements made by researchers in the field of tissue engineering; progress appears to be persisting at a rapid pace and will likely continue beyond basic research to the development of scaffolds for the creation of fully functional organs.

References

Abraham L C, Dice J F, Finn P F, Mesires N T, Lee K, Kaplan D L (2007). Extracellular matrix remodeling—methods to quantify cell-matrix interactions. Biomaterials, 28(2): 151–161

Abu-Rub M T, Billiar K L, Van Es M H, Knight A, Rodriguez B J, Zeugolis D I, McMahon S, Windebank A J, Pandit A (2011). Nano-textured self-assembled aligned hydrogels promote directional neurite guidance and overcome inhibition by myelin associated glycoprotein. Soft Matter, 7(6): 2770–2781

Agrez M V, Bates R C, Boyd A W, Burns G F (1991). Arg-Gly-Asp-containing peptides expose novel collagen receptors on fibroblasts: implications for wound healing. Cell Regul, 2(12): 1035–1044

Alvarez-Perez M A, Guarino V, Cirillo V, Ambrosio L (2010). Influence of gelatin cues in PCL electrospun membranes on nerve outgrowth. Biomacromolecules, 11(9): 2238–2246

Andukuri A, Minor W P, Kushwaha M, Anderson J M, Jun H W, Jun H W (2010). Effect of endothelium mimicking self-assembled nanomatrices on cell adhesion and spreading of human endothelial cells and smooth muscle cells. Nanomedicine, 6(2): 289–297

Anselme K (2000). Osteoblast adhesion on biomaterials. Biomaterials, 21(7): 667–681

Ayres C E, Bowlin G L, Henderson S C, Taylor L, Shultz J, Alexander J, Telemeco T A, Simpson D G (2006). Modulation of anisotropy in electrospun tissue-engineering scaffolds: Analysis of fiber alignment by the fast Fourier transform. Biomaterials, 27(32): 5524–5534

Ayres C E, Bowlin G L, Pizinger R, Taylor L T, Keen C A, Simpson D G (2007). Incremental changes in anisotropy induce incremental changes in the material properties of electrospun scaffolds. Acta Biomater, 3(5): 651–661

Barbani N, Guerra G D, Cristallini C, Urciuoli P, Avvisati R, Sala A, Rosellini E (2011). Hydroxyapatite/gelatin/gellan sponges as nanocomposite scaffolds for bone reconstruction. J Mater Sci Mater Med, (Epub ahead of print)

Barczyk M, Carracedo S, Gullberg D (2010). Integrins. Cell Tissue Res, 339(1): 269–280

Barnes C P, Sell S A, Boland E D, Simpson D G, Bowlin G L (2007). Nanofiber technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev, 59(14): 1413–1433

Barnhart E L, Lee K C, Keren K, Mogilner A, Theriot J A (2011). An adhesion-dependent switch between mechanisms that determine motile cell shape. PLoS Biol, 9(5): e1001059

Béduer A, Vieu C, Arnauduc F, Sol J C, Loubinoux I, Vaysse L (2012). Engineering of adult human neural stem cells differentiation through surface micropatterning. Biomaterials, 33(2): 504–514

Benton G, Kleinman H K, Arnaoutova I (2011). Multiple uses of basement membrane-like matrix (BME/Matrigel) *in vitro* and *in vivo* with cancer cells. International Journal of Cancer, 128(8):1751– 1757

Berndt P, Fields G B, Tirrell M (1995). Synthetic lipidation of peptides and amino acids: monolayer structure and properties. J Am Chem Soc, 117(37): 9515–9522

Bhattarai N, Edmondson D, Veiseh O, Matsen F A, Zhang M (2005). Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials, 26(31): 6176–6184

Bigi A, Panzavolta S, Rubini K (2004). Relationship between triple-helix content and mechanical properties of gelatin films. Biomaterials, 25(25): 5675–5680

Brown E J (1986). The role of extracellular matrix proteins in the control of phagocytosis. J Leukoc Biol, 39(5): 579–591

Chaubey A, Ross K J, Leadbetter R M, Burg K J (2008). Surface patterning: tool to modulate stem cell differentiation in an adipose system. J Biomed Mater Res B Appl Biomater, 84B(1): 70–78

Cheema U, Ananta M, Mudera V (2011). Collagen: applications of a natural polymer in regenerative medicine. Regenerative Medicine and Tissue Engineering—Cells and Biomaterials. Eberli D, Ed. In Tech. 287–300

Chen V J, Ma P X (2004). Nano-fibrous poly(L-lactic acid) scaffolds with interconnected spherical macropores. Biomaterials, 25(11): 2065–2073

Chen Y J, Chung M C, Jane Yao C C, Huang C H, Chang H H, Jeng J H, Young T H (2012). The effects of acellular amniotic membrane matrix on osteogenic differentiation and ERK1/2 signaling in human dental apical papilla cells. Biomaterials, 33(2): 455–463

Colognato H, Yurchenco P D (2000). Form and function: the laminin family of heterotrimers. Dev Dyn, 218(2): 213–234

Coxon A, Rieu P, Barkalow F J, Askari S, Sharpe A H, von Andrian U H, Arnaout M A, Mayadas T N (1996). A novel role for the β_2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. Immunity, 5(6): 653–666

Cybulsky M I, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos J C, Connelly P W, Milstone D S (2001). A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest, 107(10): 1255–1262

Davis G E (1992). Affinity of integrins for damaged extracellular matrix: alpha v beta 3 binds to denatured collagen type I through RGD sites. Biochem Biophys Res Commun, 182(3): 1025–1031

Duca L, Floquet N, Alix A J, Haye B, Debelle L (2004). Elastin as a matrikine. Crit Rev Oncol Hematol, 49(3): 235–244

Friedl P (2004). Prespecification and plasticity: shifting mechanisms of cell migration. Curr Opin Cell Biol, 16(1): 14–23

Friedl P, Bröcker E B (2000). The biology of cell locomotion within three-dimensional extracellular matrix. Cell Mol Life Sci, 57(1): 41–64

Friedl P, Hegerfeldt Y, Tusch M (2004). Collective cell migration in morphogenesis and cancer. Int J Dev Biol, 48(5-6): 441–449

Ghibaudo M, Trichet L, Le Digabel J, Richert A, Hersen P, Ladoux B (2009). Substrate topography induces a crossover from 2D to 3D behavior in fibroblast migration. Biophys J, 97(1): 357–368

Gobin A S, West J L (2002). Cell migration through defined, synthetic extracellular matrix analogues. FASEB J, 16(7): 751–753

Golias C, Batistatou A, Bablekos G, Charalabopoulos A, Peschos D, Mitsopoulos P, Charalabopoulos K (2011). Physiology and pathophysiology of selectins, integrins, and IgSF cell adhesion molecules focusing on inflammation. A paradigm model on infectious endocarditis. Cell Commun Adhes, 18(3): 19–32

Gonzalez A L, El-Bjeirami W, West J L, McIntire L V, Smith C W (2006). Transendothelial migration enhances integrin-dependent

human neutrophil chemokinesis. J Leukoc Biol, 81(3): 686–695

Gonzalez A L, Gobin A S, West J L, McIntire L V, Smith C W (2004). Integrin interactions with immobilized peptides in polyethylene glycol diacrylate hydrogels. Tissue Eng, 10(11–12): 1775–1786

Grinnell F (1984). Fibronectin and wound healing. J Cell Biochem, 26 (2): 107–116

Gumbiner B M (1996). Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell, 84(3): 345–357

Gumbiner B M (2005). Regulation of cadherin-mediated adhesion in morphogenesis. Nat Rev Mol Cell Biol, 6(8): 622–634

Guo L T, Zhang X U, Kuang W, Xu H, Liu L A, Vilquin J T, Miyagoe-Suzuki Y, Takeda S, Ruegg M A, Wewer U M, Engvall E (2003). Laminin alpha2 deficiency and muscular dystrophy; genotype-phenotype correlation in mutant mice. Neuromuscul Disord, 13(3): 207–215

Handley M E, Pollara G, Chain B M, Katz D R (2005). The use of targeted microbeads for quantitative analysis of the phagocytic properties of human monocyte-derived dendritic cells. J Immunol Methods, 297(1–2): 27–38

Hayashi Y, Shumsky J S, Connors T, Otsuka T, Fischer I, Tessler A, Murray M (2005). Immunosuppression with either cyclosporine a or FK506 supports survival of transplanted fibroblasts and promotes growth of host axons into the transplant after spinal cord injury. J Neurotrauma, 22(11): 1267–1281

Hughes C S, Postovit L M, Lajoie G A (2010). Matrigel: a complex protein mixture required for optimal growth of cell culture. Proteomics, 10(9): 1886–1890

Huo Y, Hafezi-Moghadam A, Ley K (2000). Role of vascular cell adhesion molecule-1 and fibronectin connecting segment-1 in monocyte rolling and adhesion on early atherosclerotic lesions. Circ Res, 87(2): 153–159

Jayaraman K, Kotaki M, Zhang Y, Mo X, Ramakrishna S (2004). Recent advances in polymer nanofibers. J Nanosci Nanotechnol, 4(1–2): 52–65

Jha B S, Ayres C E, Bowman J R, Telemeco T A, Sell S A, Bowlin G L, Simpson DG (2011). Electrospun collagen: a tissue engineering scaffold with unique functional properties in a wide variety of applications. J Nanomaterials,

Ju Y M, Choi J S, Atala A, Yoo J J, Lee S J (2010). Bilayered scaffold for engineering cellularized blood vessels. Biomaterials, 31(15): 4313–4321

Kafi A M, El-Said W A, Kim T H, Choi J W (2012). Cell adhesion, spreading, and proliferation on surface functionalized with RGD nanopillar arrays. Biomaterials, 33(3):731–739

Komoriya A, Green L J, Mervic M, Yamada S S, Yamada K M, Humphries M J (1991). The minimal essential sequence for a major cell type-specific adhesion site (CS1) within the alternatively spliced type III connecting segment domain of fibronectin is leucine-aspartic acid-valine. J Biol Chem, 266(23): 15075–15079

Koo W, Ahn S J, Zhang H, Wang J C, Yim E K F (2011). Human corneal keratocyte response tomicro and nano-gratings on chitosan and PDMS. Cell MolBioeng., 4(3): 399–410

Kundu A K, Putnam A J (2006). Vitronectin and collagen I differentially regulate osteogenesis in mesenchymal stem cells. Biochem Biophys Res Commun, 347(1): 347–357

Ledger P W, Uchida N, Tanzer M L (1980). Immunocytochemical localization of procollagen and fibronectin in human fibroblasts: effects of the monovalent ionophore, monensin. J Cell Biol, 87(3): 663–671

Lee K Y, Mooney D J (2001). Hydrogels for tissue engineering. Chem Rev, 101(7): 1869–1880

Lee S H, Moon J J, Miller J S, West J L (2007). Poly(ethylene glycol) hydrogels conjugated with a collagenase-sensitive fluorogenic substrate to visualize collagenase activity during three-dimensional cell migration. Biomaterials, 28(20): 3163–3170

Long M, Sato M, Lim C T, Wu J, Adachi T, Inoue Y (2011). Advances in experiments in modeling in micro- and nano- biomechanics: A mini review. Cell Mol Bioeng, 4(3): 327–339

Lü S H, Lin Q, Liu Y N, Gao Q, Hao T, Wang Y, Zhou J, Wang H, Du Z, Wu J, Wang C Y (2011). Self-assembly of renal cells into engineered renal tissues in collagen/Matrigel scaffold *in vitro*. J Tissue Eng Regen Med: N/A (Epub ahead of print)

Ma P X, Zhang R (1999). Synthetic nano-scale fibrous extracellular matrix. J Biomed Mater Res, 46(1): 60–72

Ma P X, Zhang R, Xiao G, Franceschi R (2001). Engineering new bone tissue *in vitro* on highly porous poly(alpha-hydroxyl acids)/hydroxyapatite composite scaffolds. J Biomed Mater Res, 54(2): 284–293

Ma Z, Kotaki M, Inai R, Ramakrishna S (2005). Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Eng, 11(1–2): 101–109

Macrae E K, Pryzwansky K B (1984). Phagocytosis of zymosan by human neutrophils. Carlsberg Res Commun, 49(2): 315–322

Maheshwari G, Brown G, Lauffenburger D A, Wells A, Griffith L G (2000). Cell adhesion and motility depend on nanoscale RGD clustering. J Cell Sci, 113(Pt 10): 1677–1686

Man S, Tucky B, Bagheri N, Li X, Kochar R, Ransohoff R M (2009). alpha4 Integrin/FN-CS1 mediated leukocyte adhesion to brain microvascular endothelial cells under flow conditions. J Neuroimmunol, 210(1–2): 92–99

Martins-Green M (1997). The Dynamics of Cell-ECM Interactions with Implications for Tissue Engineering. Principles of Tissue Engineering. Lanza R, Langer R, Chick W, Eds. R.G. Landes Company: New York. 23–46

Matter M L, Zhang Z, Nordstedt C, Ruoslahti E (1998). The α5β1 integrin mediates elimination of amyloid-β peptide and protects against apoptosis. J Cell Biol, 141(4): 1019–1030

Matthews J A, Wnek G E, Simpson D G, Bowlin G L (2002). Electrospinning of collagen nanofibers. Biomacromolecules, 3(2): 232–238

McClure M J, Sell S A, Simpson D G, Walpoth B H, Bowlin G L (2010). A three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen: a preliminary study. Acta Biomater, 6(7): 2422–2433

McCracken K W, Howell J C, Wells J M, Spence J R (2011). Generating human intestinal tissue from pluripotent stem cells *in vitro*. Nat Protoc, 6(12): 1920–1928

Meredith J E Jr, Fazeli B, Schwartz M A (1993). The extracellular matrix as a cell survival factor. Mol Biol Cell, 4(9): 953–961

Meshel A S, Wei Q, Adelstein R S, Sheetz M P (2005). Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. Nat Cell Biol, 7(2): 157–164

Miller C, George S, Niklason L (2010). Developing a tissue-engineered model of the human bronchiole. J Tissue Eng Regen Med, 4(8): 619–627

Nathan C, Srimal S, Farber C, Sanchez E, Kabbash L, Asch A, Gailit J,

Wright S D (1989). Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. J Cell Biol, 109(3): 1341–1349

Newman S L, Tucci M A (1990). Regulation of human monocyte/macrophage function by extracellular matrix. Adherence of monocytes to collagen matrices enhances phagocytosis of opsonized bacteria by activation of complement receptors and enhancement of Fc receptor function. J Clin Invest, 86(3): 703–714

Norton L W, Park J, Babensee J E (2010). Biomaterial adjuvant effect is attenuated by anti-inflammatory drug delivery or material selection. J Control Release, 146(3): 341–348

Paik D C, Saito L Y, Sugirtharaj D D, Holmes J W (2006). Nitrite-induced cross-linking alters remodeling and mechanical properties of collagenous engineered tissues. Connect Tissue Res, 47(3): 163–176

Parkhurst M R, Saltzman W M (1992). Quantification of human neutrophil motility in three-dimensional collagen gels. Effect of collagen concentration. Biophys J, 61(2): 306–315

Partin A W, Schoeniger J S, Mohler J L, Coffey D S (1989). Fourier analysis of cell motility: correlation of motility with metastatic potential. Proc Natl Acad Sci USA, 86(4): 1254–1258

Peppas N A (2004). Hydrogels. In Biomaterials Science, 2nd Edition. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, Eds. Elsevier Academic Press, 100–106

Petersen T H, Calle E A, Zhao L,Lee E J, Gui L, Raredon M B, Gavrilov K, Yi T, Zhuang Z W, Breuer C, Herzog E, Niklason L E (2010). Tissue-engineered lungs for *in vivo* implantation. Science, 329 (5991): 538–541

Pierschbacher M D, Ruoslahti E (1984). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature, 309(5963): 30–33

Pluskota E, Soloviev D A, Szpak D, Weber C, Plow E F (2008). Neutrophil apoptosis: selective regulation by different ligands of integrin alphaMbeta2. J Immunol, 181(5): 3609–3619

Ricard-Blum S (2011). The collagen family. Cold Spring Harb Perspect Biol, 3(1): a004978

Roca-Cusachs P, Gauthier N C, Del Rio A, Sheetz M P (2009). Clustering of alpha5beta1 integrins determines adhesion strength whereas alphavbeta3 and talin enable mechanotransduction. Proc Natl Acad Sci USA, 22(106): 16245–16250

Rodgers U R, Weiss A S (2005). Cellular interactions with elastin. Pathol Biol (Paris), 53(7): 390–398

Rogers T H, Babensee J E (2011). The role of integrins in the recognition and response of dendritic cells to biomaterials. Biomaterials, 32(5): 1270–1279

Roh J D, Sawh-Martinez R, Brennan M P, Jay S M, Devine L, Rao D A, Yi T, Mirensky T L, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman W M, Snyder E, Kyriakides T R, Pober J S, Breuer C K (2010). Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. Proc Natl Acad Sci USA, 107(10): 4669–4674

Rubel C, Fernández G C, Dran G, Bompadre M B, Isturiz M A, Palermo M S (2001). Fibrinogen promotes neutrophil activation and delays apoptosis. J Immunol, 166(3): 2002–2010

Ruoslahti E (1996). RGD and other recognition sequences for integrins. Annu Rev Cell Dev Biol, 12(1): 697–715

Saltzman W M, Livingston T L, Parkhurst M R (1999). Antibodies to CD18 influence neutrophil migration through extracellular matrix. J Leukoc Biol, 65(3): 356–363

Saltzman W M, Parkhurst M R, Parsons-Wingerter P, Zhu W H (1992). Three-dimensional cell cultures mimic tissues. Ann N Y Acad Sci, 665(665): 259–273

Schwarbauer JE, DeSimone DW (2011). Fibronectins, their fibrillogenesis and *in vivo* Functions. Prespectives in Biology, Cold Spring Harbor, USA

Sell S A, Wolfe P S, Garg K, McCool J M, Rodriguez I A, Bowlin G L (2010). The use of natural polymers in tissue engineering: A focus on electrospun extracellular matrix analogues. Polymers., 2(4): 522–553

Shepherd B R, Enis D R, Wang F, Suarez Y, Pober J S, Schechner J S (2006). Vascularization and engraftment of a human skin substitute using circulating progenitor cell-derived endothelial cells. FASEB J, 20(10): 1739–1741

Simon-Assmann P, Orend G, Mammadova-Bach E, Spenlé C, Lefebvre O (2011). Role of laminins in physiological and pathological angiogenesis. Int J Dev Biol, 55(4–5): 455–465

Simpson D G, Terracio L, Terracio M, Price R L, Turner D C, Borg T K (1994). Modulation of cardiac myocyte phenotype *in vitro* by the composition and orientation of the extracellular matrix. J Cell Physiol, 161(1): 89–105

Singer A J, Clark R A (1999). Cutaneous wound healing. N Engl J Med, 341(10): 738–746

Singh P, Carraher C, Schwarzbauer J E (2010). Assembly of fibronectin extracellular matrix. Annu Rev Cell Dev Biol, 26(1): 397–419

Sniadecki N J, Anguelouch A, Yang M T, Lamb C M, Liu Z, Kirschner S B, Liu Y, Reich D H, Chen C S (2007). Magnetic microposts as an approach to apply forces to living cells. Proc Natl Acad Sci USA, 104 (37): 14553–14558

Stupack D G, Cheresh D A (2004). Integrins and angiogenesis. Curr Top Dev Biol, 64: 207–238

Sugawara K, Tsuruta D, Ishii M, Jones J C, Kobayashi H (2008). Laminin-332 and -511 in skin. Exp Dermatol, 17(6): 473–480

Tan J, Saltzman W M (2002). Topographical control of human neutrophil motility on micropatterned materials with various surface chemistry. Biomaterials, 23(15): 3215–3225

Telemeco T A, Ayres C E, Bowlin G L, Wnek G E, Boland E D, Cohen N, Baumgarten C M, Mathews J, Simpson D G (2005). Regulation of cellular infiltration into tissue engineering scaffolds composed of submicron diameter fibrils produced by electrospinning. Acta Biomater, 1(4): 377–385

Thein-Han W W, Misra R D (2009). Biomimetic chitosan-nanohydroxyapatite composite scaffolds for bone tissue engineering. Acta Biomater, 5(4): 1182–1197

Todorovic V, Chen C C, Hay N, Lau L F (2005). The matrix protein CCN1 (CYR61) induces apoptosis in fibroblasts. J Cell Biol, 171(3): 559–568

Tuluc F, Garcia A, Bredetean O, Meshki J, Kunapuli S P (2004). Primary granule release from human neutrophils is potentiated by soluble fibrinogen through a mechanism depending on multiple intracellular signaling pathways. Am J Physiol Cell Physiol, 287(5): C1264–C1272

Tzu J, Marinkovich M P (2008). Bridging structure with function: structural, regulatory, and developmental role of laminins. Int J Biochem Cell Biol, 40(2): 199–214

Underhill D M (2003). Macrophage recognition of zymosan particles. J Endotoxin Res, 9(3): 176–180

van de Witte P, Dijkstra P J, Van den Berg J W A, Feijen J (1996). Phase separation processes in polymer solutions in relation to membrane formation. J Membr Sci, 117(1–2): 1–31

Varki A (1994). Selectin ligands. Proc Natl Acad Sci USA, 91(16): 7390–7397

Vasita R, Katti D S (2006). Nanofibers and their applications in tissue engineering. Int J Nanomedicine, 1(1): 15–30

Wagenseil J E, Mecham R P (2007). New insights into elastic fiber assembly. Birth Defects Res C Embryo Today, 81(4): 229–240

Wang Y Y, Lü L X, Feng Z Q, Xiao Z D, Huang N P (2010). Cellular compatibility of RGD-modified chitosan nanofibers with aligned or random orientation. Biomed Mater, 5(5): 054112

Werbowetski T, Bjerkvig R, Del Maestro R F (2004). Evidence for a secreted chemorepellent that directs glioma cell invasion. J Neurobiol, 60(1): 71–88

Wierzbicka-Patynowski I, Schwarzbauer J E (2003). The ins and outs of fibronectin matrix assembly. J Cell Sci, 116(16): 3269–3276

Wilson B D, Gibson C C, Sorensen L K, Guilhermier M Y, Clinger M, Kelley L L, Shiu Y T, Li D Y (2011). Novel approach for endothelializing vascular devices: understanding and exploiting elastin-endothelial interactions. Ann Biomed Eng, 39(1): 337–346

Woo K M, Seo J, Zhang R, Ma P X (2007). Suppression of apoptosis by enhanced protein adsorption on polymer/hydroxyapatite composite scaffolds. Biomaterials, 28(16): 2622–2630

Wu C, Fields A J, Kapteijn B A, McDonald J A (1995). The role of alpha 4 beta 1 integrin in cell motility and fibronectin matrix assembly. J Cell Sci, 108(Pt 2): 821–829

Zhang R, Ma P X (2000). Synthetic nano-fibrillar extracellular matrices with predesigned macroporous architectures. J Biomed Mater Res, 52 (2): 430–438