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Development of biocompatible synthetic extracellular matrices for tissue engineering

Byung-Soo Kim and David J. Mooney

Tissue engineering may provide an alternative to organ and tissue transplantation, both of which suffer from a limitation of supply. Cell transplantation using biodegradable synthetic extracellular matrices offers the possibility of creating completely natural new tissues and so replacing lost or malfunctioning organs or tissues. Synthetic extracellular matrices fabricated from biocompatible, biodegradable polymers play an important role in the formation of functional new tissue from transplanted cells. They provide a temporary scaffolding to guide new tissue growth and organization, and may provide specific signals intended to retain tissue-specific gene expression.

The loss or failure of an organ or tissue is one of the most severe human health problems. Treating patients with these health problems consumes approximately half of the total annual health-care costs in the USA[1]. Tissue or organ transplantation is a standard therapy to treat these patients, but this is severely limited by a donor shortage. For example, fewer than 7500 organs were available for transplantation in 1996, with approximately 50 000 patients registered on transplant waiting lists in the USA alone (United Network for Organ Sharing, 1997). Other available therapies to treat these patients include surgical reconstruction (e.g. heart), drug therapy (e.g. insulin for a malfunctioning pancreas), synthetic prostheses (e.g. polymeric vascular prostheses) and mechanical devices (e.g. kidney dialysers). Although these therapies are not limited by supply, they do not replace all functions of a lost organ or tissue and often fail in the long term[1].

Tissue engineering has emerged as a promising approach to treat the loss or malfunction of a tissue or organ without the limitations of current therapies. This approach has a foundation in the biological observation

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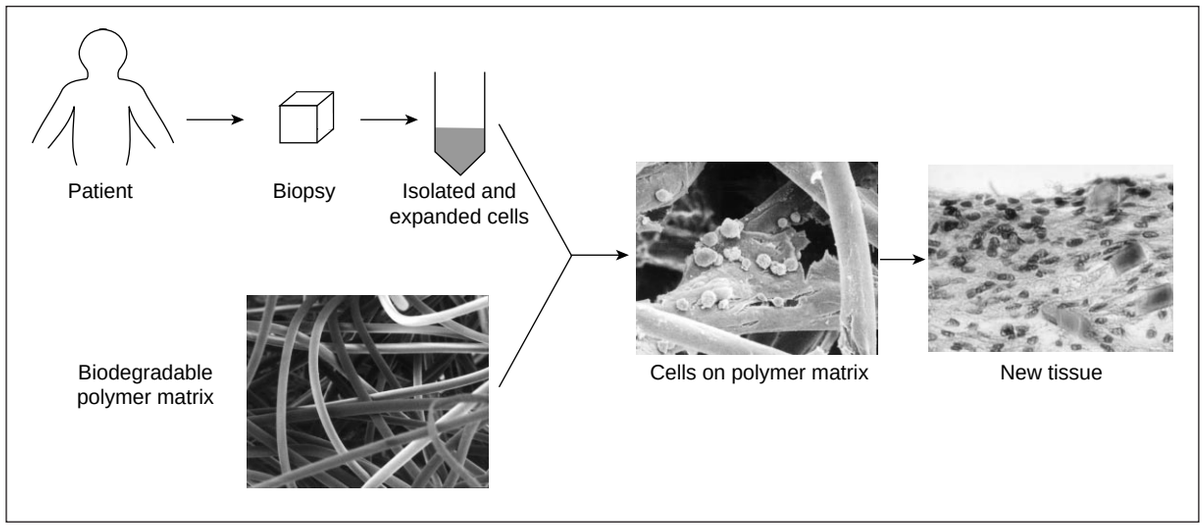

Figure 1

The tissue-engineering approach to developing organ replacements using cultured cells. Tissue-specific cells can be isolated from a small tissue biopsy and expanded *in vitro*. The cells are subsequently seeded onto open, porous, exogenous extracellular matrices fabricated from biocompatible, biodegradable polymers. A new tissue results over time and this tissue can be transplanted into a patient. Completely natural new tissues will result following polymer degradation.

that dissociated cells will reassemble *in vitro* into structures that resemble the original tissue when provided with an appropriate environment (e.g. isolated endothelial cells reform tubular structures *in vitro*[2]). Tissue-engineering approaches typically employ exogenous three-dimensional extracellular matrices (ECMs) to engineer new natural tissues from isolated cells. The exogenous ECMs are designed to bring the desired cell types into contact in an appropriate three-dimensional environment, and also provide mechanical support until the newly formed tissues are structurally stabilized and specific signals to guide the gene expression of cells forming the tissue[3]. In one approach, cells isolated from a small biopsy and expanded *in vitro* can be seeded onto a suitable exogenous ECM; they are then either allowed to develop into a new tissue *in vitro* or transplanted into a patient to create a new functional tissue that is structurally integrated with the body[1] (Fig. 1). This tissue-engineering approach may circumvent the main limitations of the conventional therapies, which include a limited supply of donor tissues and limited function of synthetic prostheses or mechanical devices. A great number of patients could be treated with a small tissue supply by expanding the isolated cells to a clinically meaningful cell mass.

One approach to designing exogenous ECMs for tissue engineering is to mimic the functions of the ECM molecules naturally found in tissues. These native ECMs act as a scaffolding to bring cells together in a tissue, to control the tissue structure and to regulate the cell phenotype (e.g. tissue-specific gene expression)[4]. In soft tissues, they consist mainly of collagens, proteoglycans and glycosaminoglycans[4]; the collagen fibres (Fig. 2) provide tensile strength, while a hydrated gel of proteoglycans fills the extracellular space, creating a space for the tissue while allowing the diffusion of nutrients, metabolites and growth factors. Molecules such as fibronectin mediate cell adhesion. The ECM may also serve as a storage depot for growth factors and provide these factors in a controlled manner to cells adjacent to the ECM[4].

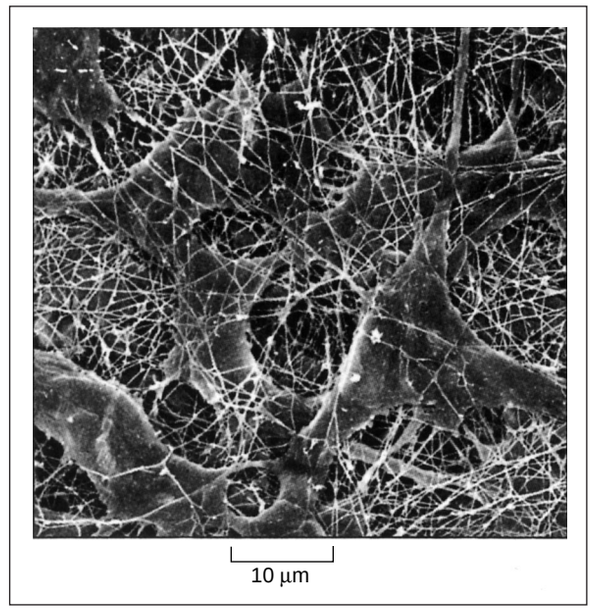

Figure 2

Scanning electron micrograph of native extracellular matrix in connective tissue. It is largely composed of collagen fibrils. The hydrogel, composed of proteoglycans and glycosaminoglycans, that normally fills the interstices of this fibrous network has been removed by the processing treatment. (Reproduced from Ref. 5 with permission from the publisher.)

In this article, we will first discuss the design requirements for synthetic ECMs in tissue engineering and then review the materials and methods that have been used to fabricate synthetic ECMs to engineer specific tissues. Design criteria for synthetic ECMs may vary considerably depending on the specific strategy utilized to create a new tissue. The focus here is on cell-transplantation approaches for creating structurally integrated tissues, not the induction of new tissue formation by host cells or by immunoisolated-cell

transplantation[1]. Significant progress has been made in the use of naturally derived ECM molecules (e.g. type-I collagen) and ceramics in specific applications, but this review will focus on approaches using synthetic polymeric materials.

Design requirements of synthetic ECMs

All synthetic ECMs used to engineer tissues have three primary roles. First, the synthetic ECMs facilitate the localization and delivery of cells to specific sites in the body. Second, they define and maintain a three-dimensional space for the formation of new tissues with appropriate structure. Third, they guide the development of new tissues with appropriate function.

Synthetic ECMs provide an adhesion substrate for transplanted cells and serve as a delivery vehicle into specific sites in the body. A large surface-area-to-volume ratio is desirable in order to allow delivery of a high density of cells, which dictates the use of highly porous matrices. It is often desirable for the new tissues to have a predefined gross morphology (e.g. blood vessels); this might be achieved by an appropriate choice of mechanical and degradative properties of the synthetic ECMs. The synthetic ECM should provide temporary mechanical support sufficient to withstand *in vivo* forces and maintain a potential space for tissue development. This mechanical support by the synthetic ECM should be maintained until the engineered tissue has sufficient mechanical integrity to support itself. The cells composing the engineered tissue must express appropriate genes to maintain the tissue-specific function of the engineered tissue. The function of seeded cells is strongly dependent on the specific cell-surface receptors (e.g. integrins) used by cells to interact with the material[6], on interactions with surrounding cells[7] and on the presence of soluble growth factors[8]. These factors can be controlled by incorporating or integrating a variety of signals, such as cell-adhesion peptides[9] and growth factors[10], into the synthetic ECM or subjecting it to mechanical stimuli[11].

To replace the functions of a lost or deficient tissue completely, the engineered tissue must be fully integrated with the host body in terms of vascular supply. Vascularization of engineered tissues is critical, as cells more than approximately 200 μm from a blood supply are either metabolically inactive or necrotic, owing to the limitation of nutrient diffusion (e.g. oxygen)[12]. Therefore, it will be necessary to promote the ingrowth of blood vessels to facilitate metabolic transport to and from surrounding native tissue if one hopes to create a large new tissue. It may be possible to control the vascularization of engineered tissue by controlling the porosity and pore size of the synthetic ECM[13] and by incorporating angiogenic factors[14]. One new approach involves the transplantation of endothelial cells in an effort to engineer a vascular network from these cells, rather than waiting for host-blood-vessel ingrowth[15]. In this approach, the engineered vasculature and ingrowing host vasculature must merge to create a functional vascular network. It is also critical to induce the innervation of engineered tissues to integrate them fully with the body; peripheral-nerve regeneration may be promoted with grafts, guidance channels and various nerve-growth factors[16].

Materials for synthetic ECMs

The exogenous ECMs for tissue engineering can be fabricated from two classes of biomaterial: naturally derived materials and synthetic materials. Naturally occurring materials, such as collagen, have the potential advantage of specific cell interactions. However, these materials are isolated from human or animal tissue, and so are typically not available in large quantities and suffer from batch-to-batch variations. In addition, naturally derived materials offer limited versatility in designing an exogenous ECM with specific properties (e.g. mechanical strength). Synthetic materials, by contrast, can be manufactured reproducibly on a large scale, and can also be processed into an exogenous ECM in which the macrostructure, mechanical properties and degradation time can be readily controlled and manipulated. Exogenous ECMs fabricated from biodegradable polymers will eventually erode in the body, avoiding a chronic foreign-body response. The greatest disadvantage of synthetic materials is, however, the lack of cell-recognition signals.

Polyesters of naturally occurring α-hydroxy acids, polyglycolic acid (PGA), poly(L-lactic acid) (PLLA) and copolymers of poly(lactic-*co*-glycolic acid) (PLGA) are widely used in tissue engineering (Fig. 3). These polymers have gained the approval of the US Food and Drug Administration for human clinical use in a variety of applications, including sutures[17]. The ester bonds in these polymers are susceptible to hydrolysis and so they degrade by nonenzymatic hydrolysis. The degradation rate of these polymers can be tailored, the process taking from several weeks to several years, by altering the copolymer ratio of lactic to glycolic acids. The degradation products of PGA, PLLA and PLGA

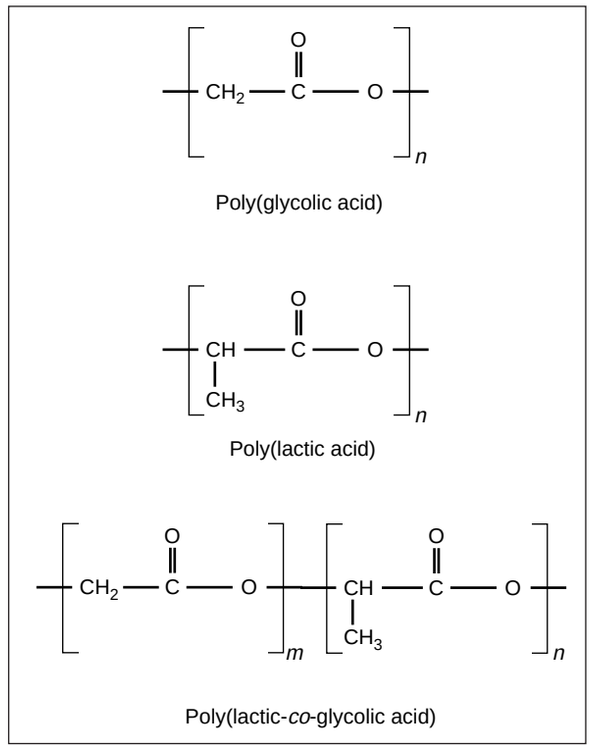

Figure 3
Biodegradable polyesters commonly used to fabricate synthetic extracellular matrices.

are nontoxic natural metabolites and are eliminated from the body via the respiratory route[17]. Because these polymers are thermoplastics, they can be easily formed into desired shapes by various techniques including moulding, extrusion and solvent casting. Although they have been widely used in a variety of biomedical applications, their acidic degradation products and lack of functional groups available for covalent modification limit their usefulness. Other biodegradable synthetic polymers, including poly(anhydrides) and poly (orthoesters), can also be used to fabricate synthetic ECMs with controlled properties[18].

Amino-acid-based polymers are also attractive candidates for the fabrication of tissue-engineering matrices. Advances in genetic engineering and fermentation technology have enabled the synthesis of entirely new artificial proteins in bacteria, including collagen-like, silk-like and elastin-like proteins[9]. Amino-acid-based polymers offer certain desirable properties (e.g. ability to interact with cells), but issues of immunogenicity and purification from contaminants during large-scale production must be addressed. Recent efforts to overcome these limitations have included the chemical synthesis of pseudo-poly(amino acid)s, such as tyrosine-derived polycarbonates or polyarylates[19].

Alginate, a polysaccharide isolated from seaweed, has been used as an injectable cell-delivery vehicle[20] and a cell-immobilization matrix[21] owing to its gentle gelling properties. Alginate is a family of copolymers of D-mannuronate and L-guluronate. It forms gels in the presence of divalent ions such as Ca^{2+}. The physical and mechanical properties of alginate gel are strongly correlated with the proportion and length of the polyguluronate block in the alginate chains[21]. The advantages of alginate include its wide availability, low diffusional barriers for nutrients and relative biocompatibility. However, alginate is not biodegradable in the human body and exhibits variations of composition and purity from batch to batch. Furthermore, gel formation is dependent on calcium ions, which can be lost following implantation.

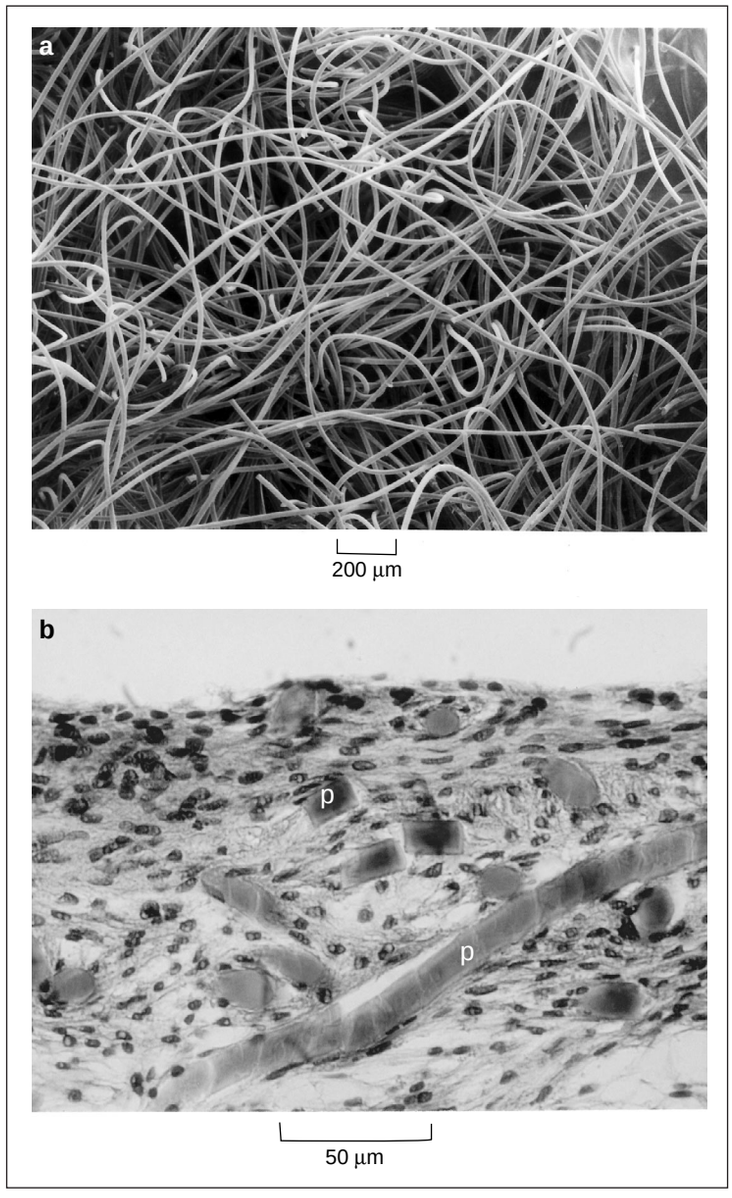

Figure 4

(**a**) Scanning electron micrograph of polyglycolic acid (PGA)-fibre-based matrix. (**b**) Photomicrograph of a cross-section of smooth-muscle tissue that resulted from four weeks growth of smooth-muscle cells on a PGA fibre-based matrix *in vitro*. The section was stained with haematoxylin and eosin. Degrading PGA fibres are visible (p), as are the cells and secreted extracellular matrix from the cells. (Reproduced from Ref. 28 with permission from the publisher.)

Fabrication of synthetic ECMs regulating gross tissue structure

Macroporous synthetic ECMs can regulate the organization of cells seeded into the matrix and the subsequent proliferation of the cells to form new tissues. A variety of processing techniques are available to fabricate synthetic ECMs from synthetic polymers, and various biodegradable polymers have been processed into a variety of configurations, including fibres, porous sponges and tubular structures.

Fibre-based scaffolds are typically composed of PGA or other crystalline polymers; PGA is readily formed into fibres (10–15 μm in diameter) by polymer extrusion. Textile-processing techniques[22] are then applied to crimp, cut and needle these fibres to form woven or nonwoven arrays with porosities up to 97% and fibre surface-area-to-volume ratios as high as 0.05 μm^{-1} (Fig. 4a). However, these scaffolds are typically incapable of resisting large compressive loads and may collapse *in vivo*[13]; however, physically bonding adjacent fibres of nonwoven PGA-fibre-based scaffolds stabilizes these scaffolds to compressive loads. One technique for achieving this involves coating fibres with a secondary polymer, typically either PLLA or PLGA[23,24], the pattern and extent of bonding being controlled by the processing parameters; thermal treatment of PGA-fibre-based scaffolds can also result in the bonding of adjacent PGA fibres[25]. The degradation rate of these stabilized scaffolds can be controlled by altering the coating polymer[17,23]. PGA-fibre-based scaffolds are easily shaped into a variety of configurations[23,24,26]. Various cell types have been cultured on these fibrous scaffolds and successfully transplanted into animal models, including chondrocytes[22,24,26], osteoblasts[27], hepatocytes[25], smooth-muscle cells[23,28], endothelial cells[23], intestinal epithelial cells[29], dermal fibroblasts[30] and urothelial cells[31] (Fig. 4b). Importantly, specific tissue architectures (e.g. tubes, ear and nasoseptum) have been

achieved by choosing the appropriate mechanical properties and degradation rate for the polymer matrix[23,24,26].

Highly porous PLLA or PLGA sponges are also widely used as structural synthetic ECMs (Fig. 5a). A variety of techniques are available for porous-sponge fabrication, including solvent casting and particulate leaching[32], phase separation[33], and gas foaming[34]. The advantage of the last method is its elimination of the need for organic solvents or high temperatures in the fabrication process, which may be especially advantageous if one wishes to deliver protein growth factors from the matrix to transplanted cells. All these fabrication techniques can yield highly porous sponges, with porosities up to 94%, that are capable of resisting larger compressive loads (approximately 10^4 Pa) than are unbonded fibre-based scaffolds (approximately 10^2 Pa), owing to the continuous solid phase[13]. Macroporous sponges have been utilized to engineer a variety of tissues, such as liver[10], cartilage[35] and bone[36], with predefined gross structures (Fig. 5b).

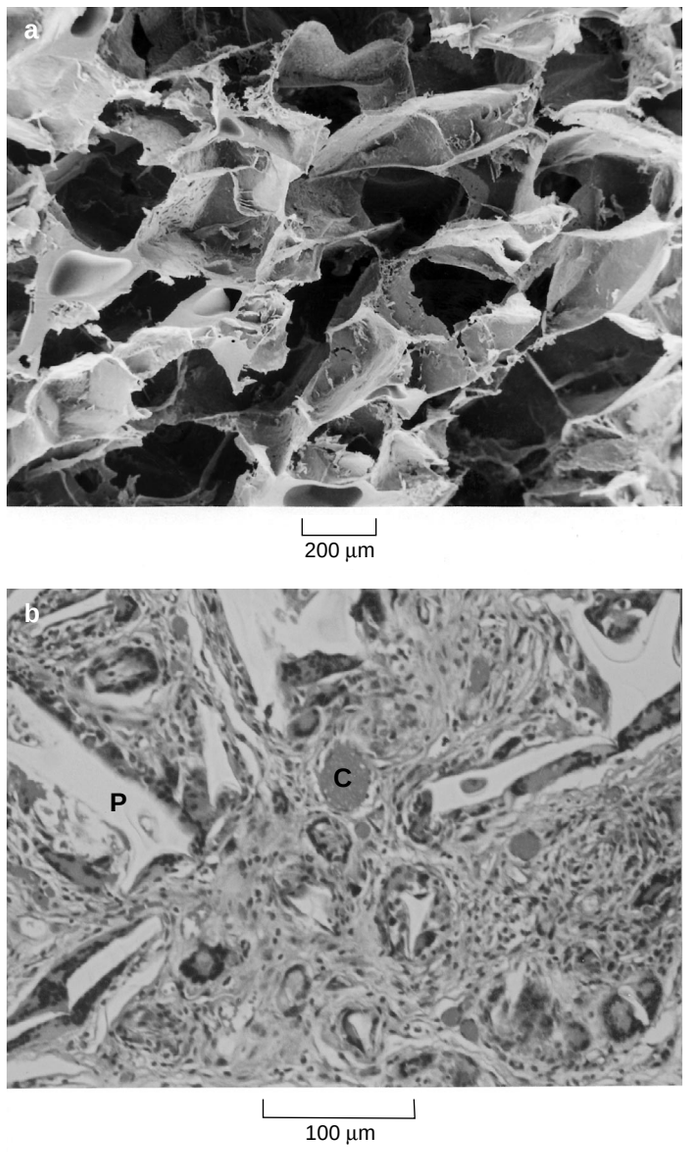

Figure 5

(**a**) Scanning electron micrograph of sponge matrix fabricated from poly(L-lactic acid) (PLLA). (**b**) Photomicrograph of cross-section of smooth-muscle tissue engineered on the PLLA sponge matrix after four weeks *in vivo*. The section was stained with haematoxylin and eosin. Polymers (P) and capillaries (C) are also visible (B-S. Kim, unpublished).

Regulating the phenotype of engineered tissues

The microenvironment of an engineered tissue must be properly regulated during the process of tissue development to induce the appropriate pattern of gene expression in cells forming the new tissue. The expression of genes by cells in engineered tissues may be regulated by multiple interactions with the microenvironment, including interactions with the adhesion surface, with other cells and with soluble growth factors, and mechanical stimuli imposed on the cells. Synthetic ECMs must provide the appropriate combination of these signals if one wishes to trigger a specific programme of gene expression. Relatively little work has been reported to date in the tissue-engineering field at this level of regulation, but this issue is critical to the development of the field.

The ECM to which a cell adheres can regulate the phenotype of the cell. For example, the density of ECM molecules *in vitro* regulates the liver-specific gene expression of cultured hepatocytes[37]. Cell adhesion to ECM is mediated by cell-surface receptors, an important class of these being the integrins[6]. Integrins function as a physical link between the cell surface and ECM, and also connect the ECM to the intracellular cytoskeleton (Fig. 6). This enables integrins to trigger a number of signalling pathways, some of which are primarily related to cell adhesion, whereas others are shared with growth-factor receptors[6]. The integrin receptors bind to relatively short amino acid sequences [e.g. Arg–Gly–Asp (RGD)] on ECM molecules. RGD-containing sequences, and many other cell-adhesion sequences, have been incorporated into biomaterials to stimulate adhesion and specific functions of cells using approaches including adsorption or covalent bonding at the surface, and covalent bonding throughout the bulk of the materials[9]. Not only must specific cell–matrix interactions be promoted, but non-specific interactions must be minimized. Towards this end, efforts are being made to incorporate cell-adhesion peptides into gel-forming polymers (e.g. alginate), which normally exhibit little cellular interaction[38]. Unlike the macroporous synthetic ECM, hydrogels completely surround the transplanted cells and define the microscopic environment of an engineered tissue. Mechanical signals conveyed to cells via their adhesion to a matrix (Fig. 6) also clearly regulate the development of various tissues and the gene expression of many cell types in culture[11]. In order to engineer functional structural tissues (e.g. bone), the correct mechanical stimuli may have to be provided during the process of tissue development via an appropriate synthetic ECM. For example, tendons engineered without mechanical stimuli do not have mechanical strength as great as native tendon, although they appear to be histologically identical[39].

Cell–cell interactions can also be exploited to regulate gene expression. For example, a significant increase in albumin synthesis can be induced in engineered liver tissues by hepatocyte aggregation[7]. The chondrocyte seeding density has also been shown to control the formation of cartilaginous tissues engineered *in vivo*[40].

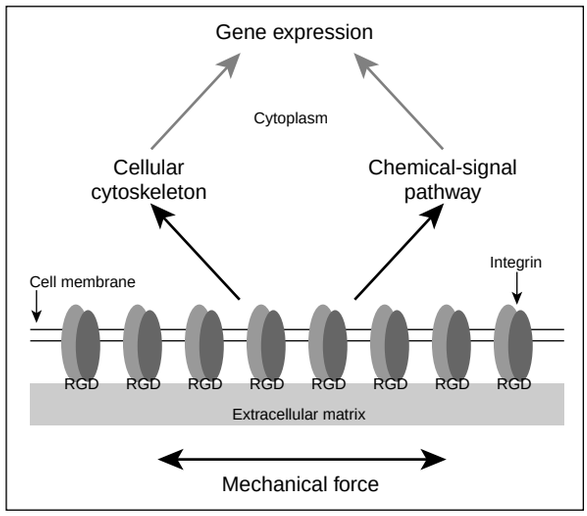

Figure 6

Schematic diagram of cell adhesion to extracellular matrix (ECM) molecules containing a specific cell-adhesion sequence [Arg–Gly–Asp (RGD)]. Cells bind to the ECM through integrin cell-surface receptors, which consist of two subunits. Mechanical forces in the ECM are transduced into the cell via integrin-mediated cell binding. The integrin-mediated cell-binding and mechanical forces influence gene expression via either cytoskeleton or chemical-signal pathways (e.g. tyrosine phosphorylation).

Polymer systems to control cell aggregation have recently been developed[41].

Soluble factors, such as growth factors and hormones, also regulate cellular phenotype, but soluble factors critical to tissue development may not be present in the site to which cells are transplanted. Controlled-drug-delivery technology is being combined with tissue engineering to provide transplanted cells with specific soluble factors in their local environment. For example, the delivery of epidermal growth factor with PLGA microspheres enhanced the engraftment of transplanted hepatocytes[10]. A variety of biologically active molecules could be delivered with this system or from the matrices described earlier, including growth factors, growth inhibitors, angiogenic factors and/or immunosuppressive agents.

Future prospects

Synthetic ECMs have proved to be suitable devices to transplant cells and guide tissue development from these cells. Although much progress has been made, many challenges still remain. The concept of combining synthetic materials with cell-recognition sites of naturally derived materials is very attractive. These hybrid materials could possess the favourable properties of synthetic materials, including widely varying mechanical and degradative properties, reproducible large-scale production and good processability, as well as the specific biological activities of naturally derived materials. This last property may be needed to engineer complex tissues with multiple cell types organized in specific patterns. Several cell-adhesion ligands with highly specific recognition could potentially be displayed spatially in a desirable pattern to induce specific cell-organization schemes. This approach has been investigated to promote endothelial cell adhesion to engineered blood vessels[9].

The processing of the chosen materials into appropriate three-dimensional matrices will be critical. Three matrix size scales may be important in controlling tissue development: the macroscopic shape of the matrix (cm to mm scale) determines the gross shape and size of the engineered tissue; the size and structure of the matrix pores (μm scale) regulate cell invasion and growth; the surface chemistry of the matrices (nm scale) controls the adhesion and gene expression of cells in contact with the matrix. Matrices with structures defined at the mm to μm scales can be designed and subsequently fabricated using computer-assisted-design–computer-assisted-manufacture (CAD–CAM) systems. For example, an ear or jaw designed exactly to meet a patient's contours could be reconstructed using magnetic-resonance-imaging or computed-axial-tomography images, and the matrices so designed could subsequently be manufactured using three-dimensional CAM or printing techniques[42]. Advances in nanotechnology[43] may ultimately allow the synthesis of materials with desirable nanoscale structures.

In summary, engineering tissues may provide an alternative to current therapies used to treat the loss or failure of tissue function. The development of synthetic ECMs is a critical component of this field and research in this area is based on the understanding of native ECM molecules (e.g. fibronectin). The matrices developed for tissue engineering may, in turn, provide a novel experimental system to elucidate the mechanisms by which native ECMs regulate tissue development.

Acknowledgments

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Negative-strand RNA viruses: applications to biotechnology

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The establishment of reverse-genetics techniques to manipulate the genome of negative-strand RNA viruses has contributed enormously to a better understanding of the replication mechanisms and pathogenicity of this group of viruses. The generation of recombinant viruses bearing specific mutations in the coding and noncoding regions of their genomic RNAs now allows the functions in the replicative cycle of specific RNA regions and protein domains of these viruses to be studied. In addition, recombinant negative-strand RNA viruses can now be designed to have specific properties that make them attractive biotechnological tools.

Despite their simplicity, viruses are sophisticated organisms that have evolved highly efficient techniques for infecting cells, expressing their genomes and generating new copies of themselves. The development of recombinant DNA techniques has made it possible to exploit these properties. It is now possible to manipulate most of the different classes of known viruses genetically, and recombinant viruses expressing novel proteins are being used as important research tools to study the structure and function of these proteins; they might also be used to deliver selected genes into higher organisms for use in gene therapy, vaccines and immunotherapy.

The negative-strand RNA viruses are a broad group of viruses that includes several important human pathogens, such as influenza, measles, mumps, respiratory syncytial, parainfluenza, rabies, Ebola and hantaviruses. All these viruses are enveloped viruses whose genome consists of either one (in paramyxoviruses, rhabdoviruses, filoviruses and Borna-disease virus) or several (in orthomyxoviruses, bunyaviruses and arenaviruses) RNA segments, and some representative viruses belonging to these groups are listed in Table 1. The virus carries its own RNA-dependent RNA polymerase, which is responsible for the transcription and replication of the viral genome in the infected cell. The genome of these viruses is found in both virions and infected cells to be complexed with the viral nucleoprotein NP or N as ribonucleoprotein (RNP) complexes, and it is these RNP complexes, rather than naked viral RNA, that are the actual templates recognized by the viral RNA polymerase. Replication involves the synthesis of a replicative intermediate consisting of a complementary copy of the genome, known as the antigenome, which is also encapsidated. The genome is also used as template by the viral RNA polymerase to synthesize the viral mRNAs, which, in contrast to the antigenomes, are capped and polyadenylated (Fig. 1). This general strategy of transcription and replication is found in all negative-strand RNA viruses and makes the generation of infectious viruses following the expression or transfection of exclusively viral RNA in mammalian cells impossible, because the

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