Chapter 1

3D Cell Culture: A Review of Current Approaches and Techniques

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Abstract

Cell culture in two dimensions has been routinely and diligently undertaken in thousands of laboratories worldwide for the past four decades. However, the culture of cells in two dimensions is arguably primitive and does not reproduce the anatomy or physiology of a tissue for informative or useful study. Creating a third dimension for cell culture is clearly more relevant, but requires a multidisciplinary approach and multidisciplinary expertise. When entering the third dimension, investigators need to consider the design of scaffolds for supporting the organisation of cells or the use of bioreactors for controlling nutrient and waste product exchange. As 3D culture systems become more mature and relevant to human and animal physiology, the ability to design and develop co-cultures becomes possible as does the ability to integrate stem cells. The primary objectives for developing 3D cell culture systems vary widely – and range from engineering tissues for clinical delivery through to the development of models for drug screening. The intention of this review is to provide a general overview of the common approaches and techniques for designing 3D culture models.

Key words: Cell culture, Bioreactor, Biomaterials, Tissue engineering, Imaging, Scaffold, Stem cells

1. Introduction

Since the advent of routine eukaryotic cell culture more than 40 years ago, the most common substrates for supporting cell growth have been made from polystyrene or glass and have taken the form of a flat two-dimensional surface (1). Thousands of published studies ranging from cancer drug screening through to developmental biology have relied on this format for the growth of adherent cells. A major criticism of these studies, however, is an assumption that animal physiology can be accurately reproduced using a cellular monolayer. Clearly, the presentation of a eukaryotic cell to a two-dimensional glass or polystyrene

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substrate is not an accurate representation of the extracellular matrix found in native tissue. As a result, many complex biological responses arising such as receptor expression, transcriptional expression, cellular migration, and apoptosis are known to differ quite significantly from that of the original organ or tissue in which they arise.

The role of a normal cell from division, through proliferation to migration and apoptosis, is an accurately controlled series of events that inherently relies on the principles of spatial and temporal organisation. The culture of cells in two dimensions is arguably far too simple and overlooks many parameters known to be important for accurately reproducing cell and tissue physiology. These include mechanical cues, communication between the cell and its matrix, and communication between adjacent cells. On the point of intercellular communication, many two-dimensional culture experiments fail to consider the interplay between different cell types, with the vast majority of cultures being of a single cell type. 2D co-cultures overcome some of these shortfalls, but are some way off in accurately reproducing cellular function observed within a tissue.

2. Three-Dimensional Cell Culture

In answer to these problems, a number of three-dimensional methods have been developed for a range of tissues where the culture environment takes into account the spatial organisation of the cell (2–5). A common goal for many of these studies is to bridge the gap between the use of whole animals at one end of the spectrum, with cellular monolayers at the other. It is therefore necessary to create a growth environment that mimics the native tissue as closely as possible, and a simple starting point is the introduction of cells into a porous biocompatible scaffold. However, the complexity of 3D systems then becomes apparent with a number of parameters to consider. Important criteria include the choice of material for the scaffold, the source of cells, and the actual methods of culture, which in practice varies considerably according to the tissue of study. A number of common approaches exist, but so too does the opinion of investigators – from the precise design of scaffolds through to the sourcing of a particular cell type. For example, does one use naturally derived or synthetic materials for a scaffold? Does one use autologous or adult-derived stem cells? Does one invest time and money fabricating an accurate nanostructured scaffold, or produce a microstructured scaffold with an approximate geometry for maintaining cell growth?

3. Three-Dimensional Culture Models

Three-dimensional culture models can be grouped into the study of whole animals and organotypic explant cultures (including embryos), cell spheroids, microcarrier cultures, and tissue-engineered models (6). Not all three-dimensional culture models require a scaffold; however, the use of scaffolds for 3D models has certainly increased considerably in the past ten years. Whole animal and organotypic explants are principally used in studies where an absolute requirement for tissue-specific information is needed (7). These models enable data where the cell is physically located within its native environment. Examples include *drosophila melanogaster* (fruitfly) and the use of zebrafish and mouse embryos. Experimental versatility in terms of environmental conditions is permissible for non-mammalian models such as the fruitfly and zebrafish, but maintaining cellular viability for mouse embryos is an absolute necessity, and so culture conditions such as pH, temperature, and O_2 levels must be very carefully controlled for these models (6, 8). Organ explantation for culture has largely been pioneered in the areas of brain and neural physiology. Here, explants can be maintained in vitro in gels or on semi-permeable membranes in the presence of an isotonic or nutrient medium. Advantages include the maintenance of tissue architecture and importantly the presence of differentiated cells within the tissue (6). Technical demands for these models include the time available for maintaining specimen integrity and the need to image deeply into samples.

Cellular spheroids are simple three-dimensional models that can be generated from a wide range of cell types and form due to the tendency of adherent cells to aggregate. They are typically created from single culture or co-culture techniques such as hanging drop, rotating culture, or concave plate methods (6, 9, 10). Spheroids do not require scaffolds and can readily be imaged by light, fluorescence, and confocal microscopy. Consequently, spheroids have seen a use in modelling solid tumour growth and metastasis studies and are also used in a multitude of therapeutic studies, e.g. for high throughput screening (11). An analogous approach is in the development of epithelial tissues to form polarised sheets, such as the epidermis of skin (12). Normal human keratinocytes can be isolated from skin and cultured on supports such as collagen gels, synthetic polymer membranes, microfibre meshes, or de-epidermalised human dermis (DED) (12). The use of DED involves removing the dermis of its original cellular components, but, importantly for 3D cell culture, it maintains many of the native basement membrane proteins (e.g. collagen type IV). The presence of these proteins in the matrix is an absolute necessity for the reconstructive adhesion and growth of keratinocytes thereafter (13).

4. Biomaterial Scaffolds for Fabricating Structure and Shape

As one increases the size and complexity of a three-dimensional model, the need for a scaffold becomes apparent. Cellular aggregates require the careful exchange of nutrients and gases in addition to spatial control, and problems with cell death arise when aggregate thicknesses of 1–2 mm arise through a lack of mass transfer, principally through a limited exchange of nutrients and waste metabolites (6, 14). This has been addressed by the use of highly porous scaffolds where basic designs consider shape, cell adhesion sites and the flow of gases, nutrients, and metabolites (4). Different cell types are embedded within matrices possessing distinctly different properties and shapes. For example, if engineering peripheral nerve, one must consider the native structure where axons are surrounded by a soft uniaxially aligned lipoprotein myelin sheath. In contrast, osteoblasts adhere to a hard surface of bone within cuboidal sheets. Consequently, the design of scaffolds must reflect the tissue of interest and a tremendous diversity exists in the design of scaffolds for the engineering of tissues (4). An important consideration is the intended application and use. Clinical work that requires a functioning implant may require just a temporary biodegradable scaffold, which after implantation is remodelled by the body and replaced by native tissue to restore original function. In this instance, the scaffold must support cell growth and differentiation, and a physical match must exist between the size of the scaffold and that of the defect. Furthermore, the scaffold should break down into metabolites without a toxic or immunogenic response. Alternatively, scaffolds may be intended as a 3D in vitro model, e.g. to further our understanding in a fundamental aspect of tissue biology or to generate systems for drug and cosmetics screening (15). Here, there is a need to accurately reproduce the native tissue structure containing cells at a given stage of differentiation, and arguably there is a greater need to image these models for cell function and response. The absolute size of the scaffold for these models and the need for hydrolysis or degradation may not be quite so important.

The choice of bulk materials to be used for scaffold fabrication includes metals, glasses, polymers, and ceramics (4). Polymers are commonly used due to an ability to control their chemical and structural properties, in combination with methods for fabrication. They are typically grouped into synthetic and natural derivatives (4). Synthetic polymers include materials such as poly glycolic acid (PGA) and poly lactic acid (PLA), whereas natural polymers include materials such as chitosan and collagen. A general requirement for all biomaterial scaffolds is to reproduce an extracellular matrix environment for supporting cell growth outside of the body.

The bulk chemical composition of a biomaterial must therefore be the first consideration when designing a scaffold, with biocompatibility being a priority for implantation (3). In particular, a material must be selected that avoids triggering an immune response or the development of a fibrous capsule. A degradable scaffold should ideally be used for clinical purposes and most degradable synthetic scaffolds such as PGA undergo hydrolysis in situ. Consequently, the body must be able to metabolise the monomeric products released during breakdown without a toxic or inflammatory response. For systems such as PGA/PLA, degradation rates can be readily tuned by the composition of PGA versus PLA, where a higher PGA content degrades faster. Natural scaffolds such as collagen are degraded by enzymolysis and consequently less control is possible on tailoring the breakdown rate. However, natural scaffolds tend to exhibit better biocompatible properties over synthetic materials – but their clinical use is concerned with potential disease transmission (4, 12), a situation avoided by the use of synthetic scaffolds.

The surface chemical properties of a biomaterial are fundamental for dictating the adhesion and spreading of living cells (16). Such properties are not necessarily governed by the bulk chemistry, in particular due to surface modification with soluble proteins derived either from the growth medium or from the cells themselves (17). Surface chemistry is predominantly controlled by charge and polarity, which in general terms control the attractiveness of proteins in solution to diffuse and adsorb at the surface. The rate at which this happens is determined by the Vroman effect, whereby highly mobile proteins in a heterogeneous mixture will reach a surface quickly, but in time may be replaced by more slowly moving proteins with a higher affinity (18, 19). This arises in particular for serum proteins, where fibrin will adsorb to a polymer surface rapidly leading to fibronectin depletion in vivo. A relationship exists between the extent of charge at a surface and the proportion of proteins that are adsorbed. This is known to correlate with the tendency of cells to adhere to a biomaterial, where the cell interacts via an adsorbed protein layer, rather than directly to the biomaterial surface (16).

Optimising the surface chemistry of biomaterials can therefore be controlled either to increase or decrease protein adsorption and in turn cellular attachment. A good example of an approach for increasing cellular attachment is given in chapter 10, where Schwann cell adherence to aligned PLA microfibres is improved by the deposition of a plasma acrylic acid layer (20). Here an increase in the negative surface charge of acid groups is associated with an increase in cell attachment and proliferation. Conversely, the deposition of allyl amine serves to prevent Schwann cell attachment. Although both layers contain surface

charges, the surface chemical groups must also dictate not just the extent of protein adsorption, but the folding conformation of the protein. At a molecular level, the interaction between acid or amine groups in the plasma polymer with amino acids containing polar, non-polar, and charged groups will dictate how a protein interacts and folds at the surface. This determines whether the adsorbed protein presents adhesive ligands permissive for binding to receptors such as integrins. A number of similar studies using plasma deposition are reported in the literature where the aim was to optimise cell adhesion and growth, e.g. the adhesion of human keratinocytes to polymer sheets for clinical delivery (21). Conversely, non-fouling surfaces such as polyethylene glycol serve to minimise protein adsorption (or fouling) and in turn cellular adhesion. The theory as to why PEG surfaces are non-fouling is highly complex – indeed the mechanisms are still being investigated. Predominant reasons suggest that chain mobility and a steric stabilising force are important, with protein-resistant properties arising through both a mixing interaction and excluded volume component (22). Thus, when a protein approaches a PEG layer, the available volume per glycol unit is decreased resulting in a repulsive force, due to a decrease in conformational entropy. In addition, the compressive force of a protein into a PEG layer reduces the total number of confirmations originally available to the chain, which creates an osmotically repulsive force, effectively pushing the protein away from the PEG layer (22).

Cell adhesion can also be controlled by integrating precise structural motifs into a biomaterial. Original work from Massia and Hubbell in 1991 reported that the alpha-V-beta-3 integrin adhesion ligand RGD, when covalently attached to a surface with a critical spacing of 440 nm, was permissive for the attachment of fibroblast cells in vitro (colloquially known as the "Hubbell limit") (23). If the separation distance between ligands was decreased to 140 nm, then fibroblast stress fibre and focal contact formation was observed. This has led many investigators to conjugate RGD-like ligands for attachment into and onto biomaterial surfaces for controlling cellular adhesion (4). However, many direct conjugation methods, while elegant, are confined predominantly to cell culture in 2D. In contrast, surface modification techniques such as plasma vapour phase deposition have proved to be effective for influencing cell adhesion in 3D scaffolds (20, 21). For example, Barry et al. report on the use of an allyl amine plasma polymer specifically for encouraging fibroblast cell attachment, morphology, and metabolic activity into 3D P(DL)LA porous scaffolds without changing the bulk characteristics of the scaffold (24). A major advantage of this approach is in the rapidity, reproducibility, and chemical control possible for modifying 3D scaffolds.

5. Scaffolds and Length Scales

The ultimate aim of a scaffold is to produce features found naturally within the extracellular matrix required for native cell function. Consequently, design criteria must consider lengthscales which span the macro-, micro-, and nanoscale. Macroscale structures are important for determining the overall size and shape of a scaffold. If constructs are for clinical purposes, then a range of shapes may be needed for implantation at a defect site. This has implications for the tissue engineering of constructs and whether they should be required as "off the shelf" products or alternatively be tailored to individual patient requirements. Bespoke products can be manufactured by modelling a defect site, e.g. a bone lesion can be characterised using computerised tomography or magnetic resonance imaging for producing a 3D macrostructure. This can be followed by computer-aided design and fabrication techniques such as stereolithography for generating a bespoke scaffold (25).

Micron length scales must be considered when reproducing tissue architectures. For example, organised parallel fibres are important for reconstructing peripheral nerve (26), while random non-woven networks may be more relevant for dermal replacement (27, 28). Irrespective of the tissue under study, particular attention should be given to parameters that control pore size, connectivity, and geometry (4). Microstructural features in general terms are important for ensuring cell adhesion, as the size of many adherent somatic cell types typically spans a distance of 10–150 μm. However, nanoscale features are being argued as more important for scaffold design (29), discussed below. Microscale features must permit a balance between scaffold porosity and the volume occupied when introducing cells for maintaining effective mass transport and nutrient exchange (30). The microstructure of a scaffold is also important for determining the overall mechanical properties. This is not only important for reproducing the properties of the native tissue, but also for withstanding experimental procedures in the generation and culture of the construct. Mechanical properties are also known to influence the function of cells contained within a construct. For example, it is well known that mechanical forces affect bone remodelling and repair. This knowledge has been applied to the exertion of mechanical forces on 3D bone scaffolds for tissue engineering (31). More recent information also reports on the direct effect of scaffold mechanics on adherent cells, where a stiffer versus a softer material can dictate the differentiation of stem cells in the absence of any externally applied force (32).

The fabrication of scaffolds with micron scale control has been possible for many years. Techniques such as electrospinning, wet spinning and sponge-like fabrication methods, such as freeze

drying and gas foaming, are reasonably commonplace (4). A number of these methods also enable nanostructured features to be made, e.g. the electrospinning of fibres can produce diameters ranging from tens of nanometres to tens of micrometres (33). However, while many of these techniques allow the creation of microstructural features and have controllable process conditions, they do not allow for ultimate control where the exact positioning of microstructural features is possible for copying the extracellular matrix. This raises an interesting point as the designs of many scaffolds for 3D cell culture are not an exact mimic, especially if the intended purpose is for implantation and where the scaffold is ultimately biodegradable. One question is therefore whether an intricately designed scaffold should be made if cell adherence, growth, and the restoration of tissue function can be achieved using a more approximately designed scaffold? Clearly, a balance needs to be established between investing considerable resource making a perfect scaffold versus the manufacture of a device that fulfils a number of basic criteria. A practical approach must be taken where the intended endpoint of the work dictates the 3D method used for getting there. This is particularly relevant if the 3D model is intended for clinical implantation.

Nanoscale features are important in determining how cells physically interact with a substrate and how they respond to it. Interactions between cell integrin receptors and adhesive ligands in native tissue arise when inter-ligand distances vary from tens to hundreds of nanometres (23). For example, collagen fibrils are typically organised across a length scale of 50–200 nm and enable the adhesive interaction of fibroblast cells (34). The precise control of these structures is determined by the primary sequence of amino acids and secondary structure of component proteins. The importance of nanoscale substrates for cell function has previously been studied in depth using model systems such as the spatial control of RGD ligands and their interaction with integrin receptors for determining cell adhesion, morphology, differentiation, and apoptosis (23, 34). Therefore, nanoscale features for scaffold design may be important. An increasing emphasis is being seen on methods for fabricating nanoscale structures (known as nanoengineering (29)) and includes the use of peptide hydrogels (35), the control of process conditions such as thermal-induced phase separation (36), the use of post-fabrication modifications (21, 24), and the incorporation of nanostructures into a matrix (37). This subject is dealt with in detail elsewhere (4), but irrespective of technique, one could argue that the ultimate scaffold will have controllability of desired features across the nanoscale, microscale, and macroscale. This is a major future challenge and not only requires an in depth understanding of the bulk and surface physicochemical properties of the scaffold, but also tremendous control and versatility over the methods for fabrication (29).

6. Bioreactors for 3D Constructs

An important consideration when moving from cells in culture as a 2D layer to a 3D construct is the maintenance of mass transport (6, 30). A limiting factor for survival concerns not only the ability to supply nutrients and oxygen, but also the simultaneous removal of waste products and metabolites. This is most readily seen for spheroids where diameters greater than 1 mm are associated with hypoxic centres containing necrotic cells, surrounded by an outer shell of living cells (38). This is known to arise directly due to nutrient starvation and metabolite toxicity. Information on spheroid models is useful in directing the design of more complex cell-scaffold constructs. As spheroids are entirely cellular, one could predict in general terms that the maximum depth for a given cellular mass within a larger nutrient-maintained construct will be of a similar order. This is therefore a major consideration in the design of all 3D culture systems whether for clinical purposes or for in vitro models. Early simple 3D culture models were based on static methods; however, the design and use of bioreactors is increasingly being integrated together with 3D culture systems and tissue engineered constructs (30). Bioreactors enable the precise and reproducible control over many environmental conditions required for cell culture. These include temperature, pH, medium flow rate, oxygen, nutrient supply, and waste metabolite removal. In addition, increasingly complex systems are being designed for the simultaneous control of seeding cells into scaffolds, and where relevant, the application of external forces to encourage differentiation and maturation. Common to many advanced systems now is the ability to maintain and monitor the environment during growth (30).

Several designs of bioreactor exist, but broadly, these can be grouped into rotating wall vessels, direct perfusion systems, hollow fibres, spinner flasks, and mechanical force systems (30). Rotating wall vessels provide continuously moving culture conditions where cell constructs are grown under low shear stress forces and enable high rates of mass transfer (39). The speed of rotation is such that forces exerted on the construct by rotation of the bioreactor ensure that constructs are in continuous free-flow. Direct perfusion systems allow the culture medium to pass through the construct (26, 40). A major advantage here is the ability to seed cells directly into the scaffold under flow conditions, which usually allows for a high seeding efficiency. The control of medium flow thereafter enables cell adhesion and growth, where a high mass transfer rate is typically achieved throughout the entire construct. Hollow fibre systems are used for cells that have a high metabolic rate (41). Cells are usually seeded within a matrix or scaffold contained within porous fibres. The medium is

then perfused externally over the fibres to increase mass transfer. A consideration when designing these systems is the porosity of the scaffold and whether the entire scaffold experiences metabolite exchange or just the periphery. Spinner flasks can be used to seed cells into constructs and also culture them thereafter (38). Seeding is conducted by the introduction of cells into the medium and their perturbation by the spinner, generating convection currents. Mass transfer for subsequent culture is maintained by the spinner mechanism. Mechanical force systems exploit the mechanism by which tissues respond to force during growth (42). Cells such as osteoblasts are known to be mechanoreceptive and respond to force with the activation of intracellular signal transduction pathways (43). Secondary messenger signals arising can control gene expression and determine the expression of differentiation genes thereafter, and consequently enhance conditions for 3D construct maturation (43). Bioreactors can therefore be exploited by using physiological loading regimes for determining the optimum conditions for exerting and detecting forces on a construct (42).

A future direction of bioreactor design is in the reproducible and automated production of tissues, where temperature, pH and oxygen levels are monitored and controlled simultaneously. Taking this one step further, monitoring a developing tissue by non-invasive methods such as 2-photon microscopy, MRI, or CT scanning could assess the extent of cellular growth and differentiation, allowing for flexibility in variations expected during development. While some way off, state-of-the art bioreactors such as the Advanced Clinical Tissue Engineering System (ACTES™) are in development (reviewed in (38)). ACTES™ systems are intended to be based within hospitals whereby an automated closed loop system takes a patient biopsy, isolates and expands the cells, seeds them on to a scaffold, and then cultures them until formation of a mature tissue graft. It is proposed that such systems could carry out autologous tissue grafting on site, eliminating the need for expensive GMP facilities and minimising operator handling. However, the most important aspects for bioreactor design at present address conditions for ensuring a reproducible and controlled growth environment for constructs that are millimetres to centimetres in size.

7. The Source of Cells for 3D Models

The source of cells for 3D cultures and tissue engineering usually requires a host or a donor-derived origin. A remarkable number of possibilities exist in principle for the various sites and sources, but in general terms for tissue engineering, these can be grouped into stem cells, autologous cells, allogenic cells, and xenogenic cells. For 3D in vitro models, this list can be extended to include

animal-derived primary cells, cell lines, and genetically modified variants of all the above cell types. Many investigators favour the use of autologous cells for clinical implantation, principally for the avoidance of immune rejection. A widely publicised early example was the generation of engineered cartilage (44). Here, a small healthy biopsy of cartilage was taken, the cells explanted in the laboratory, expanded, and seeded on to PGA meshes and PLA scaffolds before being implanted into the defect site. However, autologous cells are not always available and even if they are, they may not be viable or capable of proliferation in vitro. As an alternative, it is possible in some cases to use allogenic cells; however, the potential for immune incompatibility must be considered. Xenogenic cells can be used if the clinical requirement is for the supply of chemicals within a tissue. A good example of xenogenic transplantation is the use of pancreatic islets for insulin production (45). It is, however, necessary to physically contain such cells within a semi-permeable encapsulating membrane.

A commonly encountered problem with the need for primary cells is a lack of availability or an insufficient potential to generate sufficient numbers for clinical purposes. Therefore, the use of progenitor and multipotent stem cells holds great promise. Remarkable advances have been made in the isolation, expansion, characterisation, and targeted differentiation of progenitor cells towards a number of different lineages. The number of tissue sites from which haematopoietic, mesenchymal, or neural stem cells can be isolated includes the lung, liver, retina, pancreas, cardiovascular system, brain, spinal chord, adipose tissue, and bone marrow. Irrespective of this large potential source, a particular challenge for any application is the ability to direct cellular differentiation with great accuracy towards an intended phenotype. Variations are observed between the implantation of stem cells in vivo versus differentiation potential in vitro, e.g. neural stem cells have a greater range of expression markers following surgical implantation, compared to expansion of the same progenitor population in vitro (46, 47). Similarly, work on embryonic stem cells shows variations in response to stimuli such as the addition of growth factors in vitro, where not all cells differentiate equally (48). In contrast, recent work on the introduction of hECs in scaffolds followed by implantation shows extensive differentiation towards a functioning tissue, e.g. in vessel formation (49).

8. The Commercial Promise of 3D Culture

The potential to repair and restore tissue function by the clinical delivery of tissue engineered constructs sparked the creation of a number of biotechnology and healthcare companies in the 1990s.

The majority of products introduced were for the treatment of skin and epithelial injuries. However, a number of financial difficulties were encountered shortly after, not because the products were necessarily ineffective, but because of difficulties in getting the products from bench to clinic. Tissue engineered constructs can be notoriously expensive to manufacture and, in combination with needing to recover R&D costs, a very real threat is that such products are simply unaffordable to the consumer. In practice, the National Health Service in the UK predominantly determines whether a commercial product succeeds or fails. The situation in the United States is somewhat different, with the prevalence of private healthcare. Thus, cost is important for the fundamental design of tissue engineered products and arguably this starts with the basics of scaffold design, before considering if one needs to use autologous cells or stem cells. However, cost is not the only issue. A compounding factor in a number of countries is also the lack of clear regulatory guidance on facilitating the smooth transition of constructs from the laboratory to the clinic. A number of problems encountered over the past ten years have also been hampered by regulatory uncertainty, largely due to tissue-engineered products not fitting neatly into traditional forms of healthcare therapy such as devices or drugs (12).

9. A Combinatorial Approach

In summary, 3D culture models can only succeed by combining a number of key areas, in particular materials science, cell biology, bioreactor design, and aligning these to clinical applications and regulatory practice if intended for implantation. While the ultimate goal might be to create an identical tissue ex vivo, many strategies have made tremendous gains by focussing on a single aspect such as biomaterial design, an appropriate cell source, or the bioreactor environment. For tissue engineering, a more common approach has not been to make an exact copy of living tissues, but to generate a "nucleating environment" in which 3D structures have sufficient information for permitting cellular adhesion, proliferation, and differentiation into a mature and functioning construct. For example, epithelial–dermal sheets can be readily fabricated for skin reconstruction using microstructured fibre scaffolds (27, 28), questioning the need for nanostructured scaffolds. Conversely, the alignment of peripheral nerve axons for repairing traumatic injuries may require a scaffold with nanostructured features, due to the complexity of organising thousands of axons over several millimetres (26). Considerable effort is presently being invested on establishing methods for integrating cells into scaffolds, and investigating exactly how complex this

environment needs to be for promoting the formation of new tissues. Much attention has surrounded the subject of 3D culture models and tissue engineering over the past decade, and immediate clinical and commercial expectations have frequently been unrealistic. However, during this time tremendous advances have been made in the basic development of 3D models. The aim of this book is therefore to provide an overview of the methods and techniques successfully devised for practising 3D cell culture.

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