3-D Culture Systems for Cell Culture Technology

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Abstract

Many three dimensional (3D) models currently in practice, however, require expensive equipment, large sample volumes, long incubation times and/or extensive expertise, and the most disadvantages of them is that they are too far from the nature of human organs. Because of the above problems, research and development on drug discovery, regenerative medicine, biotech and pharmaceutical Industries are very costly and takes several years to bring a single drug/product to the marketing. 3D models are based on merger of biomaterials science, nanotechnology, and biological principles in order to mimic organ/tissues to partially reduce the amount of in vitro and in vivo animal testing, clinical trials, and to solve the above problems.

Conventional 3D Biomaterials Technology

The proposed technique of cell culture in three dimensional (3D) artificial materials is based on the use of 3D fibrous scaffold to guide cell organization. In comparison with conventional two dimensional (2D) cultures, cells maintained in 3D culture more closely resemble the in vitro situation with regard to cell shape and cellular environment that can influence the behavior of cells. It has been recognized that induction of tissue regeneration based on tissue engineering can be achieved by the following three key steps: the proliferation of cells, the seeding of cells and proliferation in a suitable scaffold, and the maintenance of the differentiation phenotype of the engineered tissues [1]. The property of scaffold material for cell attachment is one of the major factors contributing their morphology, proliferation, functions, and the subsequent tissue organization [2]. At first, cells attach to the material surface of scaffold, then spread, and proliferate. The 3D scaffold can provide larger surface area available for cell attachment and spreading than 2D systems (i.e., tissue culture plate). Xie et al. [3] have reported that the initial rate of cells growth was higher for the 2D culture, but once the cells reached confluent, their proliferation stopped. However, the cells growth in the 3D scaffold was continued for longer time periods than that of 2D scaffold. Other reports have demonstrated that cell proliferation was superior in the 3D scaffold than the 2D one [4]. Regenerative medicine is an interdisciplinary field that combines engineering and live sciences in order to develop techniques that enables the restoration, maintenance or enhancement of living tissues and organs. Its fundamental aim is the creation of natural tissue with the ability to restore missing organ or tissue function, which the organism has not been able to regenerate in physiological conditions. With that, it aspires to improve the health and quality of life for millions of people worldwide and to give solution to the present limitations: rejections, low quantity of donors, etc [5]. Tissue engineering needs scaffolds to serve as a substrate for seeding cells and as a physical support in order to guide the formation of the new tissue [6]. The majority of the used techniques utilize three-dimensional polymeric scaffolds, which are composed of natural or synthetic polymers. Synthetic materials are attractive because their chemical and physical properties (e.g. porosity, mechanical strength) can be specifically optimized for a particular application. The polymeric scaffolds structures are endowed with a complex internal architecture, channels and porosity that provide sites for cell attachment and maintenance of differentiated function without hindering proliferation. Ideally, a polymeric scaffold for tissue engineering should have the following characteristics: 1) To have appropriate surface properties promoting cell adhesion, proliferation and differentiation, 2) To be biocompatible, 3) To be highly porous, with a high surface area/volume ratio, with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste, 4) To have mechanical properties sufficient to withstand any in vivo stresses [7]. The last requisite is difficult to combine with the high porosity in volume of the material. That is why it is necessary to use polymeric matrices with special or reinforced properties, especially if the polymer is a hydrogel. Among many materials currently used as cell scaffolds, collagen has been widely used. The in vivo safety has been proven through the long-term applications to clinical medicine, cosmetics, and foods. The collagen sponge fabricated by freeze-drying method, followed by cross-linking of combined dehydrothermal, glutaraldehyde, and Ultraviolet (UV) is highly porous with an interconnected pore structure, which is effective in the infiltration of cells and supplying oxygen and nutrients to the cells or excluding the cells wastes, while the shape and bioresorbability can be readily regulated by changing the formulation conditions. However, as shown in Figure 4, the drawback of collagen sponge as a scaffold for cell proliferation and differentiation is its poor mechanical strength. To overcome the inherent material problem of sponge, the combination with other materials has been attempted. Considering implantation, the materials to be combined should be also bioabsorbable. From the viewpoint of clinical application, it is preferable to select the material that has been clinically used. Several biodegradable synthetic polymers, such as poly(glycolic acid) (PGA) and its copolymers with L-lactic acid, DL-lactic acid, and ε-caprolactone, have been fabricated into the cell scaffolds of non-woven fabric and sponge shapes for tissue engineering. The mechanical resistance of the scaffolds to
compression is practically acceptable for the tissue engineering applications because of their hydrophobic nature. However, the cell attachment to the surface of synthetic polymer scaffolds is poor compared with that of collagen. PGA has been approved by U.S. Food and Drug Administration for the clinical applications. Our previous study revealed that incorporation of PGA fiber enabled a collagen sponge to increase the resistance to compression in vitro and in vivo [8]. The in vitro culture experiment revealed that the number of MSC attached increased with the incorporation of PGA fiber to a significantly high extent compared with that of the original collagen sponge. It is a key for the present technology to fabricate mechanically strong collagen sponges by incorporating the PGA fiber of which the amount is as low as possible. Since collagen is more compatible to cells than PGA, at a higher amount of PGA fiber incorporated, the fiber may cause inflammation response to the sponge. Moreover, the collagen sponge does not become strong enough to resist the compressed deformation only by increasing the extent of cross linking. Because the PGA fiber incorporation also suppressed the shrinkage of collagen sponge, it is possible that the volume available for cell attachment was larger, resulting in a higher number of cells attached. This phenomenon also can be explained in terms of suppressed shrinkage of sponge by PGA fiber incorporation. The collagen sponge mechanically reinforced by PGA fiber incorporation is a promising scaffold for tissue regeneration. The incorporation of PGA fiber enabled the sponge to increase the resistance to compression. On comparing in vivo degradability, the collagen scaffold is generally digested faster than the PGA fabric. This degradation profile greatly depends on the cross-linking extent of collagen sponge and the molecular weight of PGA and the formulation shape. In our study, a combined cross-linking method of dehydrothermal, glutaraldehyde, and UV was used to prepare collagen sponges with or without PGA fiber incorporation. Weadock et al. [9] have evaluated the physical, mechanical, and biological behaviors of collagen sponge cross-linked by physical (UV irradiation and dehydrothermal) and chemical (carbodiimide and glutaraldehyde) or combination of physical (dehydrothermal) and chemical (carbodiimide). The results revealed that combination of physical (dehydrothermal) and chemical (carbodiimide) cross-linking of collagen reduced significantly swelling ratio, increased the collagenase resistance time and low and high strain modulus compared with a single cross-linking of UV, dehydrothermal, and carbodiimide. The glutaraldehyde cross-linking itself showed the same physical and mechanical properties as combination of physical (dehydrothermal) and chemical (carbodiimide) cross-linking. The polymeric scaffold design depends on the regarded applications, but in any case it must achieve structures with the aforementioned characteristics, which are necessary to their correct function. To achieve it with success is conditional on two factors: materials used, both the porogen, and the reticulate polymer, which is infiltrated in the porogen to become a scaffold; and, as a second factor, the structural architecture, both external and internal, basically shown by its porosity (high surface area/volume ratio), geometry, size pore and having in mind that the structures must be easily processed into three-dimensional. On basis of the extensive range of polymeric materials, different processing techniques have been developed to design and fabricate 3D scaffolds for tissue engineering implants [10]. They include: a) Phase separation, b) Gas foaming, c) Fiber bonding, d) Photolithography, e) Solid Free Form (SFF), f) Solvent casting in combination with particle leaching. However, none of the techniques have achieved a suitable model of tree-dimensional architecture so that the scaffolds can fulfill their aims in the wanted way, using equipments with high cost even, for the reasons that are going to be discussed. So, using phase separation, a porous structure can be easily obtained by adjusting thermodynamic and kinetic parameters. However, because of the complexity of the processing variables involved in phase-separation technique the pore structure cannot be easily controlled. Moreover, it is difficult to obtain large pores and may exhibit a lack of interconnectivity [11]. Gas foaming has the advantage of room temperature processing but produces a largely non-porous outer skin layer and a mixture of open and closed pores within the center leaving incomplete interconnectivity. The main disadvantage of the gas foaming method is that it often result in a non-connected cellular structure within the scaffold. Fiber bonding provides a large surface area for cell attachment and a rapid diffusion of nutrients in favor of cell survival and growth. However, these scaffolds, as the ones used to construct a network of bonded Polyglycolic Acid (PGA), lacked the structural stability necessary for in vivo use. In addition, the technique does not lend itself to easy and independent control of porosity and pore size.

**Advanced Techniques to Create 3D Systems**

Photolithography has been employed for patterning, obtaining structures with high resolution, although this resolution may be unnecessary for many applications of patterning in cell biology. In any case, the disadvantage of this technique is the high cost of the equipment need limits their applicability [12]. SFF scaffold manufacturing methods provide excellent control over scaffold external shape, and internal pore interconnectivity and geometry, but offer limited micro-scale resolution. Moreover, the minimum size of global-pores is 100 μm. Additionally, SFF requires complex correction of scaffold design for anisotropic shrinkage during fabrication. Moreover, it needs high cost equipments. Solvent casting in combination with particulate leaching method, which involves the casting of a mixture of monomers and initiator solution and a porogen in a mold, polymerization, followed by leaching-out of the porogen with the proper solvent to generate the pores, is inexpensive but still has to overcome some disadvantages in order to find engineering applications, namely the problem of residual porogen remains, irregular shaped pores, and insufficient interconnectivity [13]. The proposed scaffolds may find applications as structures that facilitate either tissue regeneration or repair during reconstructive operations [14]. The new structure could also find applications in other areas in which the pore morphology may play an essential role, such as membranes and filters [15,16]. In the USA alone, each year over 10,000 newly injured people are added to the total of more than 250,000 which are confined to their wheelchair [17]. A major limitation in treating nerve injury, Central Nervous System (CNS) and Peripheral Nervous System (PNS) is the failure of current therapies to induce nerve regeneration. Unfortunately, for Central Nervous System (CNS) injury, and particularly spinal cord injury there is currently no treatment available to restore nerve function [18]. One possible avenue for remedying this situation is to artificially engineer nerve tissue. It is commonly accepted that physical guidance of axons is a vital component of nerve repair. Many materials have been used in an attempt to physically guide the regeneration of damaged nerves. It has been shown that preferential alignment of channel pores may provide a unique advantage in certain medical applications, such as nerve regeneration [19]. Highly oriented Poly Lactic Acid (PLA) scaffold for spinal cord regeneration and demonstrated that highly oriented macroporous have shown to have efficiency in axonal
regeneration both in the peripheral and central nervous system. Cell migration and angiogenesis were observed and the expected orientation of axonal growth, as well. The axons were perfectly aligned along the pore direction, which confirmed the crucial role of three dimensional polymer structures [20]. It has been demonstrated that three dimensional sponges of Poly-Hydroxy Ethyl Methacrylate (PHEMA) sponges are able to house a purified population of glial cells and provided a scaffold for regenerative growth of axons in the lesioned rat optic tract may be a candidate for use as prosthetic bridges in the repair of the damaged Central Nervous System (CNS). However, they deduce that further work is necessary to optimize their procedure, like providing a more oriented trabecular network within the hydrogel scaffold [21]. Macroporous foams with size of 100 µm were produced in the form of channels by the solid-liquid phase separation technique for nerve regeneration. Nerve regeneration can only occur through a structure of interconnected pores of ideal diameter in the range of 10 to 100 µm [22]. Poly(D,L-lactide) foams have been developed with macroporous of 100 µm organized longitudinally were prepared by freeze-drying technique for spinal cord regeneration. They showed that the parallel assembly of rods of porous (diameter ~100 µm) containing an amphiphilic copolymer was a promising strategy to bridge a defect in the spinal cord of adult rats and they confirmed a high density of cells in the surface of porous interconnected structures as well [23].

Future Prospect

The promise of cell culture in 3D environment has recently been enhanced by major advances in the area of medical therapy, tissue engineering and regenerative medicine [24–40]. There are many thoughts still to be clarified regarding the application of 3D culture technology for human diseases treatment. Novel hydrogels comprised of natural and biodegradable materials with improved mechanical properties in which cells can be encapsulated are one the promising 3D technology to deliver cells into target tissue. Various scaffolding biomaterials are widely applied in tissue engineering. 3D Scaffolds have been used for increasing the rate of transplantation and collaboration of administered cells with host niche in a three-dimensional mode and are in the core of stem cells technology.

References


