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REVIEW ARTICLE

Past, present, and future of microcarrier-based tissue engineering

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Summary The top issue in tissue engineering is how to obtain more seed cells quickly and to preserve their characteristic morphology during *in vitro* expansion culture of cells. Microcarriers can help to amplify cell numbers and maintain the appropriate phenotype for tissue repair and restoration of function. In addition, microtissue with cell microcarriers can be used to repair diseased tissues or organs. This review introduces the materials used for, and classification of, microcarriers and the improvements in, and potential applications of, microtissues with cell microcarriers in tissue engineering.

Introduction

Tissues and organs can be damaged by, or become dysfunctional through, inflammation, trauma, and degeneration. Traditional therapies for restoring diseased tissues and organs using autografting or allografting are not always satisfactory. Autografting is performed at the expense of using healthy tissue from elsewhere in the body, and therefore, the source of donor organs is limited and complications and additional damage can occur. Medication and temporary replacement therapy can resolve the organ dysfunction, but is not available to every patient.

In the 1980s, researchers proposed the concept of tissue engineering, which revolutionized the treatment of tissue defects and organ failure [1]. The most-studied problems in tissue engineering include how to obtain sufficient numbers of cells with the original phenotype, how to prepare the best scaffold, and how to load factors into a scaffold to achieve the long-term controlled release of factors; these can be considered the seeds, soil, and fertilizer, respectively. Cells cultured *in vitro* in conventional monolayers can lose their characteristic morphology, and the cell-specific extracellular matrix (ECM) secretion can be altered. For example, chondrocytes can develop into spindle-shaped fibroblast-like cells that secrete fibrous tissue or fibrocartilage [2,3]. Traditional two-dimensional

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monolayer cultures can lose their phenotype during passaging [4]. High cell-density cultures favour the maintenance of the cell phenotype and prevent dedifferentiation. Cells under three-dimensional (3D) culture are stimulated mechanically, enabling them to retain a stable phenotype [5]. Because most seed cells are adherent, good adhesion on the microcarrier for growth and maintenance of differentiation and function *in vitro* for constructing tissue-engineered tissues is the key technology, especially for large-scale engineering of skin, bone, cartilage, and tendons, [6,7,8,9]. The traditional static culture method has many disadvantages, such as a small specific surface, the inhomogeneous diffusion of nutrients and metabolites, and slow cell growth affecting the final production [5].

In 1967, van Wezel [10] used the first microcarrier, diethylaminoethyl—Sephadex A50. Subsequently, it has played an important role in the proliferation of anchorage-dependent cells in the large-scale cultivation of animal cells. The technology developed rapidly in the 1980s and achieved marked success. Since then, the commercialization of microcarriers has increased and commercial microcarriers include Hillex, Glass Coated, Plastic Plus Coated, Rapid Cell P, Cytodex-3, Cytodex-2, and Cytodex-1. The material nature, surface properties, and microcarrier particle size are important factors affecting cell adhesion and proliferation. To improve the performance of the microcarrier in order to obtain better adhesion and proliferation, the microspheres are specially treated. The greatest benefit of microcarrier culture is that the microtissues formed by cells and microcarriers can be delivered to the sites of defects, thereby eliminating the digestion of cells before transfer in monolayer culture from flasks [11].

Here, we give an overview of microcarrier culture technology, its importance as an *ex vivo* research tool, and its potential application *in vivo*. Microcarriers are a promising culture system for producing great quantities of cells and microtissue for tissue engineering.

Microcarrier materials

Depending on the source, the materials used as microcarriers can be divided into two categories, namely, synthetic and natural polymers. Early microcarriers were mostly constructed from synthetic polymers, such as polyhydroxyethylmethacrylate, polystyrene, polyacrylamide, polyurethane foam, and glucose. Although microcarriers made from synthetic polymers showed good reproducibility and mechanical properties, they lacked cell recognition sites and affected cell adhesion and growth [12]. Therefore, researchers are now using natural polymers and their derivatives as the material of choice because they are easily obtained, biocompatible, and inexpensive [9,13]. Here, we review various natural polymers that were used to prepare microcarriers.

Gelatin is produced from collagen by mild, irreversible degradation. It has good biocompatibility and is relatively inexpensive. The presence of keratin, elastin protein gelatin, melanocytes, and chondroitin are important in promoting cell growth and adhesion [14]. Microcarriers made of many other substrates, such as Cytodex-3 and CT-3, are often encapsulated with a layer of gelatin to improve their biocompatibility. Commercial gelatin microcarriers include GELIBEAD (Hazelton Research Products, Reston, Virginia, USA), Ventregel (Ventrex Laboratories, Portland, Maine, USA), and CultiSpher (HyClone, Loagen, Utah, USA).

Collagen is a biological material that can be used for guided tissue regeneration, as it is nonantigenic, has good biocompatibility, and works in the tissue-healing process. Some specific sites of the polypeptide in the primary structure of denatured collagen combine with fibres in the culture medium to form a collagen—fibre complex that contributes to cell adhesion and growth [15]. Widely used in animal cell cultures, Cytodex-3 (Pharmacia) and the porous Microsphere (Verax) use collagen as a substrate. Collagen has been used to coat the surfaces of microcarriers; Hong et al. [16] produced an injectable scaffold for cartilage regeneration using a collagen-coated polylactide microcarrier/chitosan hydrogel composite. The cell metabolic activity increased rapidly with the chondrocyte/composite scaffold.

Cellulose is a homopolymer of D-glucose connected by β-1,4-glycosidic bonds. Cytopore is a commercial fibrin microcarrier [17] that has high mechanical strength and can be recycled.

The biocompatible chitin and its derivative chitosan promote wound healing, are antibacterial, and have other biological functions. Chitosan is the product of chitin deacetylation and its molecular chains are linked by hydrogen bonds. The glycosidic bonds confer rigidity and stability to the molecule; the hydrogen bonds are electropositive; the acetyl groups are hydrophobic; and the hydroxyl groups are hydrophilic, although chitosan is not soluble in water. Chitin and its derivatives are biological materials and they can also be used as cell culture scaffolds in tissue engineering [18,19].

Alginic acid is widely used as a fixing material for the drug-controlled release of proteins, cells, and DNA. It can be absorbed with no adverse reactions. Cartilage cells cultured in an alginate carrier can synthesize an ECM similar to that of natural cartilage [20].

The ECM consists mainly of polysaccharide, protein, and proteoglycan macromolecules that are synthesized and secreted by animal cells. These compounds are located on the cell surfaces or between the cells [21]. They form a complex network and support and connect the organizational structure, and regulate tissue and cell physiological activities. The ECM is part of the animal tissue and does not belong to any cell. It determines the characteristics of the connective tissue and plays an important role in some animal cells. Even *in vitro*, ECM plays a crucial role in the cell culture process. It provides a similar microenvironment to that *in vivo*, which is better for the growth of cells. Many tissue-engineering studies have investigated ECM [9,22].

Classification of microcarriers

Based on their physical characteristics, microcarriers can be divided into two main categories, namely, solid and liquid microcarriers.

Solid microcarriers have advantages in terms of the adherence and expansion of cells (Table 1). The Cytodex series is widely used. Solid microcarriers are prepared by