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Cell sheet approach for tissue engineering and regenerative medicine

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ABSTRACT

After the biotech medicine era, regenerative medicine is expected to be an advanced medicine that is capable of curing patients with difficult-to-treat diseases and physically impaired function. Our original scaffold-free cell sheet-based tissue engineering technology enables transplanted cells to be engrafted for a long time, while fully maintaining their viability. This technology has already been applied to various diseases in the clinical setting, including the cornea, esophagus, heart, periodontal ligament, and cartilage using autologous cells. Transplanted cell sheets not only replace the injured tissue and compensate for impaired function, but also deliver growth factors and cytokines in a spatiotemporal manner over a prolonged period, which leads to promotion of tissue repair. Moreover, the integration of stem cell biology and cell sheet technology with sufficient vascularization opens possibilities for fabrication of human three-dimensional vascularized dense and intact tissue grafts for regenerative medicine to parenchymal organs.

Contents

1. Introduction

Current medical treatment is based on biotech medicine supported by biogenetics and the development of physiologically active substances. This treatment contributes to prolongation of the life span and improvement in quality of life of patients. Examples of this situation include insulin, which is indispensable for type 1 diabetes. Molecular target drugs, including small compounds and antibodies, are widely used for some types of chronic inflammatory diseases and cancers. However current medicine is in the range of symptomatic treatment. Regenerative medicine, in which cells or tissues are applied as medicine, is highly expected to cure patients with difficult-to-treat diseases and physically impaired function. Development of technology to enable transplanted cells to be engrafted for a long time is indispensable for maximizing the effects of regenerative medicine. Unfortunately, the transplantation of cells as a single cell suspension is still widely used for various diseases worldwide, possibly owing to their convenience. Many cells are known to be lost soon after transplantation, which leads to marginal effects. Tissue engineering is a promising strategy to overcome these problems. Many researchers worldwide have started investigating biodegradable scaffolds. On the other hand we have developed an original scaffold-free cell sheet-based tissue engineering technology by new cooperative and integrative systems, which apply to disciplines including medicine, biology, engineering, and pharmacy [1–14]. The principle of cell sheet engineering is as follows: Poly(*N*-isopropylacrylamide) (PIPAAm), a temperature-responsive polymer,

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and its copolymer show a hydrophobic state at 37 °C, and reversibly change to the hydrophilic state below 32 °C. The temperature-responsive culture dishes have been developed by modification of cell culture surface with PIPAAm. The surface of these dishes is hydrophobic, and cells adhere and proliferate at 37 °C. By lowering the temperature to below 32 °C, the surface reversibly changes to the hydrophilic state, and cells cannot adhere to the surface because of rapid hydration and swelling of the grafted PIPAAm. This enables collection of a viable monolayer cell sheet with full preservation of the cell–cell contacts and extracellular matrices [1,2]. Therefore, this strategy can be used to yield a non-invasive harvest of cultured cells as an intact layer cell sheet containing deposited extracellular matrices. These matrices can be collected in a non-enzymatic process by simply reducing the culture temperature to below 32 °C for less than 1 h [3,4] (Fig. 1A).

For preparation of effective temperature-responsive culture dishes, various modification methods for coating PIPAAm onto substrates have been investigated. First, a thin PIPAAm hydrogel layer was modified on polystyrene dishes or glass substrate by electron beam (EB) irradiation-induced polymerization to obtain temperature responsive cell culture substrates [1,15,16]. PIPAAm layer thickness is important for thermally modulated cell adhesion and detachment, because PIPAAm hydrogel layer thickness determines the hydrophilicity of the modified PIPAAm [15,16]. Therefore, for precisely controlling PIPAAm layer thickness, living radical polymerization, which enables accurate control of polymerization, has been introduced as a PIPAAm modification method. One effective living radical polymerization for PIPAAm modification to substrates is atom transfer radical polymerization (ATRP) [17–19]. The ATRP method allows for accurate control of polymerization of PIPAAm, resulting in the precise modulation of PIPAAm chain length on the substrates. Additionally, the high graft density of PIPAAm, referred to as “polymer brush”, can be formed on substrates because of the high initiation efficiency of the ATRP-initiator. Furthermore, PIPAAm density on substrates can be modulated by changing the modified ATRP initiator density. Another type of living radical polymerization for modification of PIPAAm on substrates is reversible addition-fragmentation chain transfer (RAFT) polymerization. This polymerization method can also allow for control of polymer chain length and density on the substrate [20]. Additionally, the end functional group of the modified PIPAAm chain is useful for enhancing cells' adhesion [21] or modulation of the phase transition temperature of the modified PIPAAm [22]. On the other hand, as a facile method for fabricating thermoresponsive cell culture dishes, coating of PIPAAm-based block copolymer with hydrophobic segment, poly(*n*-butyl methacrylate)-*b*-PIPAAm, on tissue culture polystyrene dishes has been investigated [23]. Polymer layer thickness determining cell adhesion and detachment properties can be modulated by the concentration of the casting copolymer solution. Additionally, stripe patterned PIPAAm modified surfaces, consisting of cell adhesive and non-adhesive region, have been investigated for fabricating oriented cell sheets [24] and rapid cell sheet recovery [25]. In this way, many types of PIPAAm modified cell culture substrates have been developed depending on the cellular intrinsic adhesion and detachment properties, resulting in the fabrication of various kinds of cell sheets.

One of the desired mechanisms of regenerative medicine is the replacement of injured or lost tissues with appropriate cells or tissues (Fig. 1A). To date, thin-layered tissues, such as the cornea [5], esophagus [6], articular cartilage [8], and periodontal tissue [9], are fully regenerated in the preclinical and clinical settings by cell sheet transplantation of autologous mucosal epithelial cells (for cornea and esophagus), chondrocytes (for cartilage), and periodontal ligament-derived cells (periodontal tissue). Transplanted cell sheets not only replace injured tissue, but also compensate for impaired function when implanted in the ectopic region. Examples of this process include hepatic cell sheets in subcutaneous tissue, which contribute to albumin production and drug metabolism [11], and pancreatic cell sheets, which attenuate high glucose concentrations in type 1 diabetes models [12]. Recent progress in stem cell biology and reprogramming technology [26] will enable creation of patient-own cells in a scalable manner to enable

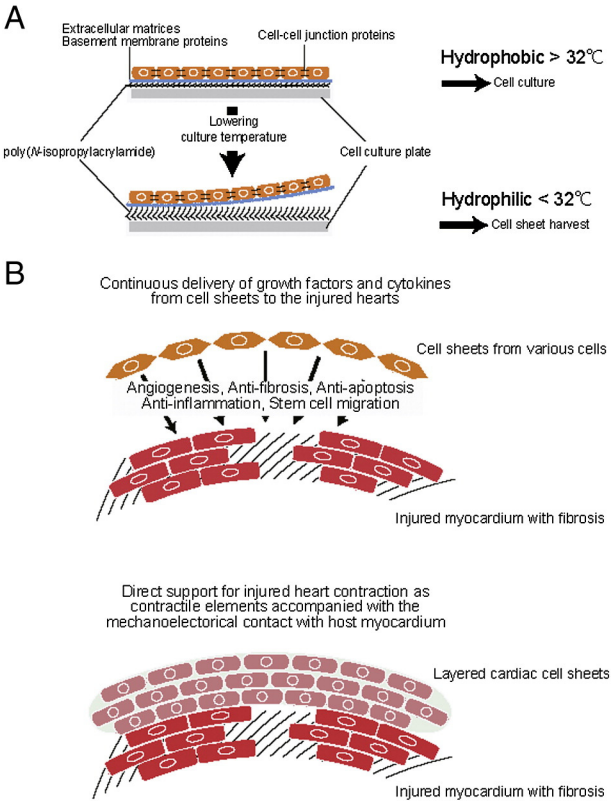

Fig. 1. Schematic illustration of cell sheet engineering (A) and possible mechanisms of cell sheet transplantation to injured hearts (B).

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