Review

Cell sheet engineering utilizing intelligent materials for regenerative medicine

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In order to realize true regenerative medicine, we have developed a novel technlogy for the reconstruction of tissues and organs by utilizing intelligent materials. Cells are cultured on temperature-responive culture surfaces at 37°C, and harvested as transplantable cell sheets by reducing temperature. With these cell sheets we regenerate various kinds of tissues including cornea and heart.

Keywords : Temperature-responsive Surfaecs, Tissue Engineering

I. INTRODUCTION

Less than 10 years ago, tissue engineering was thought of as one of the most promising fields, ready to revolutionize modern medicine.[1] However, traditional approaches using biodegradable scaffolds have so far only shown limited success.

To reconstruct various tissues such as heart or liver, it is necessary to create cell-dense structures that resemble the native architecture. In theory, the use of biodegradable scaffolds is appropriate, as polymers can be molded into the desired shape and upon degradation; the space formerly occupied by the scaffold can be filled by dividing cells and extracellular matrix (ECM). However, the deposition of large amounts of ECM can lead to structures that do not resemble native tissues, and implantation and incomplete degradation of scaffolds, can cause inflammation that can damage transplanted cells.

Another obstacle has been harvesting of cultured cells. Generally, proteolytic treatments are needed to release cells, but this also causes degradation of cell surface proteins, which are vital to cell-to-cell and cell-to-ECM interactions. To solve both problems, we developed temperature-responsive culture dishes.[2,3] These dishes are created by the grafting of the temperature-responsive polymer poly(*N*-isopropylacrylamide) onto ordinary tissue culture dishes and allow for the control of cell adhesion by simple temperature changes. At 37°C, the surface is relatively hydrophobic allowing for cells to attach, spread and proliferate similar to on normal culture dishes. However, when the temperature is reduced below 32°C, the surface becomes hydrophilic and swells, allowing cells to spontaneously detach from the surface without the need for enzymatic treatments (Fig. 1).

With this method, cells are harvested as intact sheets along with their deposited ECM.[4] Thus, tissue regeneration with cell sheet engineering can be accomplished either by transplantation of single cells sheets, as with skin, cornea, and periodontal ligament, or by homo- and hetero-typic layering of multiple cell sheets to create 3-D structures, such as cardiac muscle or liver lobules. Because deposited ECM is

maintained on the basal surface, cell sheets can adhere directly to host tissues and other cell sheets allowing for the creation of cell-dense tissue structures without any carrier substrates or scaffolds.[5]

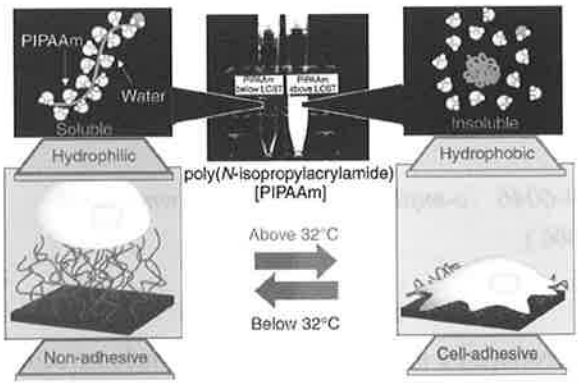

Fig.1. Temperature-responsive culture dishes. The temperature-responsive polymer poly (*N*-isopropylacrylamide) (PIPAAm) exhibits a transition from hydrophobic to hydrophilic across its lower-critical solution temperature (LCST) of 32°C. After electron-beam polymerization and grafting to normal tissue-culture polystyrene (TCPS) dishes, temperature-responsive culture surfaces can be produced. The non-invasive harvest of various cell types as intact sheets, along with deposited extracellular matrix, can be achieved by reducing the culture temperature.

II. EXAMPLES

II-A. Cornea

Corneal epithelial stem cells are located in the limbus, the transitional zone between the cornea and the peripheral conjunctiva. Trauma due to chemical and thermal burns or severe diseases can lead to limbal stem cell deficiencies which result in corneal opacification with accompanying loss of vision. lthough corneal transplantation can be used, a lack of donor corneas as well as the need for immunosuppression limits its applicability.

In cases of unilateral disease, a small biopsy from the patient's healthy eye allows for the isolation of limbal stem cells that can be expanded on temperature-responsive dishes (Fig. 2).[6] Additionally, in the cases of bilateral disease, small specimens of autologous oral mucosa can be used as the source of epithelial stem cells.[7] Stratified epithelial sheets are non-invasively harvested with deposited ECM, and transplanted without the need for carrier substrates. Our cell sheets are less fragile compared to those harvested by dispase treatment, and adhere directly to the host corneal stroma without any sutures. In all cases, a clear, smooth corneal surface was maintained, with significantly improved visual acuity. In ocular applications, the use of scaffolds or carrier substrates should be strictly avoided as their degradation can interfere with the required optical transparency and cause micro-trauma to the ocular surface.

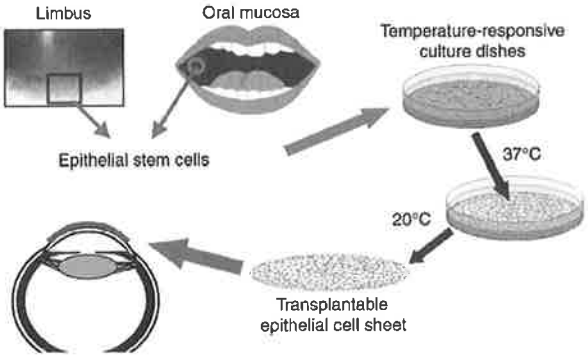

Fig.2. Corneal surface reconstruction. Small biopsies from the limbus (the border between the cornea and neighboring conjunctiva) or from oral mucosa provide for the isolation of epithelial stem cells. Cell sheets fabricated on temperature-responsive culture dishes can be harvested and transplanted directly to the ocular surface without the need for carrier substrates or sutures.

II-B. Heart

In native heart tissue, interactions between cardiomyocytes are required for spontaneous and synchronous pulsation. Scaffold-based designs can lead to the deposition of large amounts of ECM, which can prevent direct cell-to-cell interactions that are necessary for cardiac pulsation. Layered cardiomyocyte sheets harvested from temperature-responsive dishes, pulsate simultaneously and shown diffuse gap junction

formation.[8] When transplanted into the subcutaneous tissues of nude rats, spontaneous beatings could be macroscopically observed after 3 weeks and maintained for over 1 year. Histology of excised tissues also showed the formation of microvascular networks throughout the graft, characteristic of native tissues.[9] It is thought that these layered constructs can be directly attached to ischemic myocardium via their own ECM, repairing host tissues (Fig. 3).

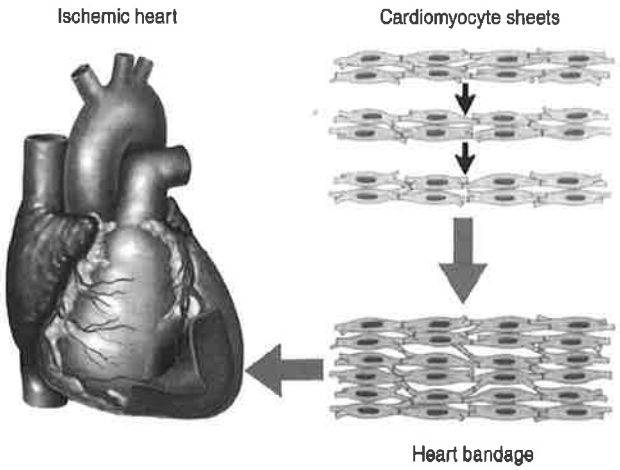

Fig.3. Myocardial cell-sheet engineering. Cardiomyocyte sheets harvested temperature-responsive culture surfaces can be layered to form three-dimensional that beat synchronously and simultaneously.We believe that layered cardiomyocyte can act as a "heart bandage" for the recovery of ischemic cardiac tissue.

Poor vascularization remains a major obstacle in bioengineering cell-dense tissues, limiting the viable size of constructs due to hypoxia, nutrient insufficiency, and waste accumulation. Therefore, new technologies for fabricating functional tissues with a well-organized vasculature are required. The thickness limit for layered cell sheets in subcutaneous tissue was ~80 μm (3 layers). To overcome this limitation, repeated transplantation of triple-layer grafts was performed at 1, 2, or 3 day intervals.[10] The two overlaid grafts completely synchronized and the whole tissues survived without necrosis in the 1 or 2 day interval cases. Multistep transplantation also created ~1 mm thick myocardium with a well-organized microvascular network. Furthermore, functional multilayer grafts fabricated over a surgically connectable artery and vein revealed complete graft perfusion via the vessels and ectopic transplantation of the grafts was successfully performed using direct vessel anastomoses.

These cultured cell sheet integration methods overcome long-standing barriers to producing thick, vascularized tissues, revealing a possible solution for the clinical repair of various damaged organs, including the impaired myocardium.

III CONCLUSION

Although the field of tissue engineering has made significant advances during the past 20 years, there still remains considerable difficulty in recreating many tissues and organs because of the limitations of traditional scaffold-based methods. We believe that cell-sheet engineering, which utilizes temperature-responsive intelligent surfaces, will overcome the problems that have limited conventional approaches in the past and establish a new basis for regenerative medicine.

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