

Chapter 12

Cell Detachment for Engineering Three-Dimensional Tissues

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Abstract Dynamic control of the biointerface between adherent cells and materials may provide a promising approach for the detachment and manipulation of cells *in vitro*. Thermoresponsive, electroresponsive, photoresponsive, pH-responsive, and magnetic systems have been reported as mechanisms for such control. These systems have been utilized to detach specific cells in a spatially controlled manner and to assemble cellular building blocks such as cell sheets and spheroids to engineer three-dimensional tissues and organs. Because assembled and thicker tissues require vascular networks to supply oxygen and nutrients throughout the constructs, some of these systems have also been employed to fabricate vascular structures in engineered tissues. This chapter provides an overview of the current technological advancements in the dynamic control of the biointerface, with particular emphasis on tissue engineering applications. A major focus of this chapter is on the application of electrochemistry to cell detachment and to engineering vascular structures. Current challenges and future prospects of these systems have been discussed.

Keywords Cell detachment · Electrochemistry · Microelectrode array · Cell sheet · Spheroid · Vasculature

12.1 Introduction

The ability to dynamically control the cell adhesive properties of a substrate has recently been shown to be powerful approaches that may foster advances in diverse fields, ranging from cell biology to tissue engineering [1, 2]. Dynamic substrate surfaces are useful for the fabrication and manipulation of cellular building blocks,

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such as cell sheets and spheroids that are used for engineering tissues and organs *in vitro* [3, 4]. Dynamic control of cell adhesion has been demonstrated with external stimuli such as thermal, magnetic, optical, and electrical triggers [5]. For example, cells on a substrate modified with a thermoresponsive polymer were detached simply by reducing the temperature from 37 to 20 °C [6]. Corneal and epithelial cell sheets stacked with this approach have been employed for the clinical treatment of corneal disorders and esophageal ulceration, respectively [7, 8]. A magnetic force has been used to attract magnetically labeled cells to a non-adherent surface. Subsequently, a cell sheet was harvested by removing the magnetic force [9]. Photoreponsive molecules, such as those containing 2-nitrobenzyl groups, were cleaved by exposure to light of a certain wavelength, typically in the ultraviolet range, which led to the detachment of cells from a substrate along with the desorption of the molecules [10]. In electroresponsive systems, electroactive molecules, such as quinone esters and O-silyl hydroquinone, that can be reductively or oxidatively cleaved by applying electrical potentials, were used to release both molecules and cells from an electrode surface [11]. In this chapter, these approaches have been categorized by their applications to spatially resolved single-cell detachment, formation and collection of spheroids, stacking of cell sheets, and engineering vascular structures.

12.2 Spatially Resolved Cell Detachment

Dynamic cell detachment technologies using external stimuli are often characterized by spatial resolution, temporal resolution, and reversibility. Thermal and magnetic approaches typically possess high temporal resolution and reversibility. However, because it is difficult to produce a large difference in thermal and magnetic fields in a limited space, these approaches may not be applicable to selective detachment of a single cell. In contrast, although optical and electrical approaches sometimes lack reversibility, they provide spatial and temporal resolutions because localized photoexposure and fabrication of microelectrode arrays can be achieved with submicron resolution.

Several studies have investigated cell detachment based on electrochemical reactions that use electrically responsive molecules on the surface of an electrode [12, 13]. Some electrochemical techniques have been used to release cells from a specific region. Marksich's group fabricated a micropattern of alkanethiol self-assembled monolayers (SAMs) terminated with three different electroactive moieties that exhibit different responses to electrical potentials applied on a gold surface. The terminal moieties captured and subsequently released the cell adhesion ligand (RGD) in response to either reductive or oxidative potential, which led to the stepwise release of specific cells attached on different electrode array patterns [14]. In another approach, desorption of alkanethiol SAMs from a gold electrode was used to detach cells from a substrate. The gold-thiolate bond was reductively cleaved by the application of a negative electrical potential to a gold surface, leading to the detachment

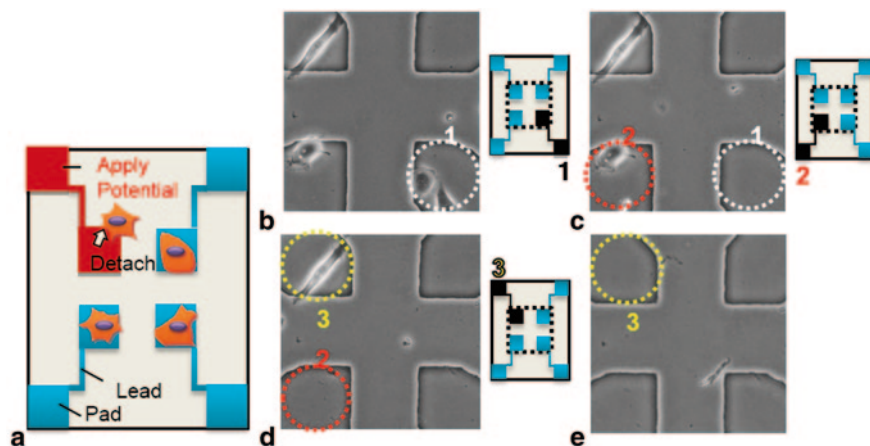


Fig. 12.1 Spatially resolved detachment of single cells using micropatterned electrodes. **a** Illustration of micropatterned ITO electrodes modified with alkanethiol SAMs. A cell on an electrode was detached by applying a negative potential. **b** The *circle* labeled 1 shows the initially activated electrode. **c** The cell on the electrode labeled 1 was detached. The *circle* labeled 2 indicates the subsequently activated electrode. **d** The cell on the electrode labeled 2 was detached. The *circle* labeled 3 indicates the activated electrode. **e** The cell on the electrode labeled 3 was detached

of cells along with the desorption of SAMs [13]. Selective detachment of cells using this technique was achieved by micropatterning electrode arrays on a glass surface (Fig. 12.1a) [15]. Instead of gold electrodes, transparent indium tin oxide (ITO) electrodes were used. Halation makes it difficult to observe cells on gold electrode arrays because of the large difference in light permeability between uncoated and gold-coated regions of glass. Cells on ITO micropatterns were individually detached with single-cell resolution (Fig. 12.1b). These techniques might be promising tools for collecting specific cells based on shape and location, a capability that is beyond typical cell sorting techniques, such as fluorescent-activated cell sorting and magnetic-activated cell sorting.

12.3 Formation and Collection of Spheroids

Multicellular aggregates such as spheroids have been investigated as cancer models for drug development and as cellular building blocks for tissue engineering applications. Compared with conventional monolayer culture, spheroid cultures are considered to provide microenvironments more closely related to *in vivo* conditions [16, 17]. For example, primary hepatocytes and articular chondrocytes maintained their organ-specific functions when they were cultured as spheroids but not when grown in monolayer cultures [18]. Various spheroid fabrication techniques that employ nonadherent surfaces [19], spinner flasks [20], hanging drop methods [21], micropatterning of cell-adhesive/non-adhesive surfaces [22, 23], and micromolding [24, 25] have been reported. Microwells consisting of thermoresponsive hydrogel

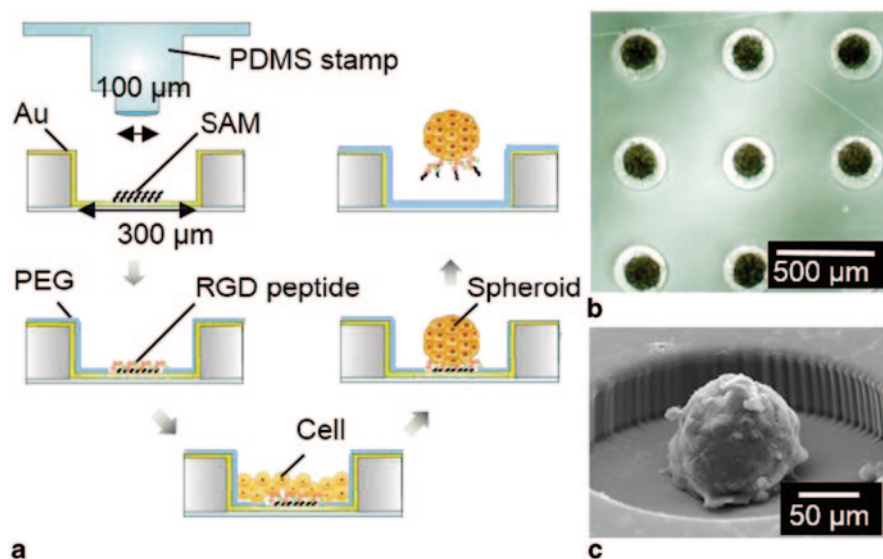


Fig. 12.2 Fabrication and collection of spheroids through an electrochemical reaction. **a** Schematic diagram. **b** Hepatocytes forming spheroids with a uniform diameter in each microwell. **c** Scanning electron microscope (SEM) image of a spheroid in a microwell

have been used not only to generate spheroids but also to subsequently release them via a temperature-induced change in the hydrogel [26]. Spheroids have been harvested also with an electrochemical approach [13]. The technique combined an electrochemical reaction with photolithography and micro-contact printing, as shown in Fig. 12.2a. Microwells were fabricated with photolithography and covered with a thin gold layer. Then, the central region of the microwells was modified with RGD peptide-terminated alkanethiol SAMs using microcontact printing. The entire region, except the alkanethiol spots, was modified with poly(ethylene glycol) to prevent cell attachment. After 3 days in culture, cells seeded in the microwells had spontaneously formed spheroids on the cell-adhesive region at the center of the microwells (Fig. 12.2b, c). The spheroids were subsequently collected by applying a negative potential. In the field of bioprinting, spheroids have been printed as an ink on a substrate in order to fabricate larger cellular tissues. These approaches to fabricating and collecting uniform spheroids might become useful tools for bioprinting and other cellular build-up approaches.

12.4 Detachment and Stacking of Cell Sheets

The most promising approach for cell sheet engineering is the use of thermoresponsive polymers [27]. Cell sheet engineering using this approach has become sophisticated in recent years and has been evaluated in diverse clinical trials, including

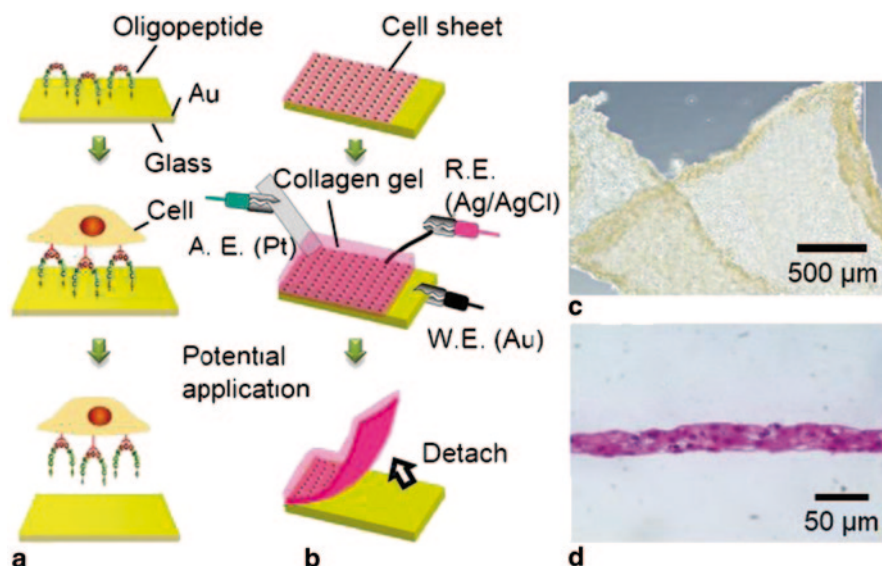


Fig. 12.3 Stacking of cell sheets by electrochemical stimuli. **a** Electrochemical detachment of cells along with the reductive desorption of the oligopeptide. **b** Detachment of cell sheets. **c** Electrochemically harvested mono-layer fibroblast sheet. **d** Three-layer hepatocyte sheet fabricated by stacking harvested cell sheets one by one

diseases of the cornea, myocardium, and esophagus. The major advantage of cell sheet engineering is that intact cell sheets can be obtained in which cell-cell junctions and deposited extracellular matrices are maintained, in contrast to their destruction during typical cell collection using trypsin. Detached cell sheets can be readily stacked with other cell sheets in succession to form a multilayered cell sheet [13]. In addition to thermoresponsive polymers, other stimulus-responsive surfaces have been proposed for engineering stacked cell sheets [13, 28–30]. Polyelectrolyte films have been used as sacrificial layers. Polyelectrolytes spontaneously adsorb onto oppositely charged substrates through electrostatic interactions and form thin polymer films. Change in the local pH at the substrate/film interface, which was caused by water electrolysis, led to dissolution of the polyelectrolyte film and detachment of cell sheets [31].

Using an electroresponsive system (Fig. 12.3a, b), similarly to the thermoresponsive system, cell sheets with the dimensions of 10 mm × 10 mm were collected (Fig. 12.3c) and stacked to fabricate multi-layered cell sheets (Fig. 12.3d). Compared to the thermoresponsive system, an advantage of the electroresponsive system is that cell sheet detachment can be accomplished more quickly. Typically, cell sheet harvesting took 30–70 min [32] with the thermoresponsive system, but took only 10 min in the electroresponsive system [13]. To improve the supply of oxygen and nutrients to dense cell sheets, polydimethylsiloxane (PDMS) membranes [24, 33] or cell culture inserts have been used [34]. PDMS possesses excellent biocompatibility and gas permeability properties [35]. Hepatoblastoma cells adhered,

grew, and formed cell sheets with a thickness of $>50\ \mu\text{m}$ on a PDMS substrate [33], primarily because of the improved oxygen supply. Further increase in the thickness of a cell sheet requires that microvessels supply oxygen through perfusion. Cocultivation of endothelial cells in cardiac cell sheets has shown that endothelial cells form vascular networks in cell sheets, thereby making it possible to fabricate thicker cardiac cell sheets [36].

12.5 Engineering Vascularized Tissues

Successful clinical applications have been limited to thin or avascular tissues, such as skin, cartilage, and bladder [37, 38], and fabricating viable 3-dimensional constructs remains a major challenge in tissue engineering. One of the major limiting factors is the inability to deliver nutrients and oxygen via vascular networks that are fundamental in most important organs in the body such as the liver, kidney, and lung. The shortage of oxygen supply poses the risk of hypoxia and necrotic cell death in the core of the tissue constructs [39].

A number of studies in recent decades have reported the fabrication of vascularized tissues *in vitro*, and endothelial cells have played a pivotal role in the formation of functional vascular networks [40]. Most previous approaches, however, relied on self-organization of endothelial cells in hydrogels and cell sheets [41, 42], which were typically too small to perfuse culture medium throughout tissue constructs in order to supply oxygen and nutrients. Thus, even when luminal structures with endothelial cells were fabricated, the supply of oxygen and nutrients was mostly limited to molecular diffusion [43]. Fabricating perfusable vascular networks might be vital for the fabrication of thick and cell-dense tissue constructs *in vitro* [44–46].

More recently, several approaches for the *in vitro* fabrication of perfusable endothelialized vascular structures have been reported [47–50]. In these approaches, endothelial cells were seeded into previously formed microchannels in hydrogels. Sufficient oxygen and nutrients for cells encapsulated in the hydrogels were provided by perfusion of culture medium through the endothelialized channels. One drawback of such approaches is that the flow of culture medium was stopped for a while in order to allow endothelial cells to attach to the inner surface of the channel, thereby making it difficult to avoid hypoxic injury to cells embedded at a high cell density in hydrogels. Prompt initiation of culture medium flow will be required to satisfy their oxygen demand.

One promising feature of electrochemical approaches is that cells can be rapidly detached from cylindrical rods to fabricate endothelial cell-lined vascular-like structures (Fig. 12.4a) [51]. By applying a negative potential to the gold rods for 5 min, endothelial cell monolayers were transferred onto the internal surface of microchannels in collagen gel. After extracting the rods from the gel, culture medium was perfused immediately. In the subsequent perfusion culture, it was seen that the transferred endothelial cells sprouted and formed luminal structures in the gel

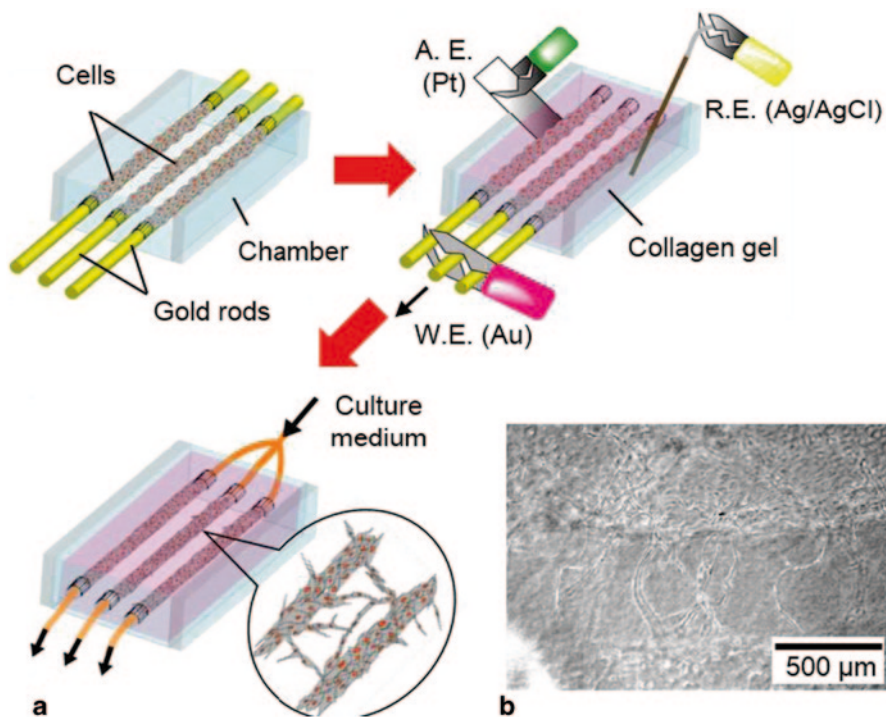


Fig. 12.4 Fabrication of vascular like structures. **a** The procedures. The oligopeptide was chemically adsorbed onto gold rods via gold–thiolate bonds. Endothelial cells attached to the surface were detached during the reductive desorption of the oligopeptide, and then the rods were extracted from the chamber. The capillaries were connected to a microsyringe pump in order to perfuse culture medium. **b** Migration of transferred endothelial cells in collagen gel. The luminal structures of endothelial cells extended to the neighboring vascular-like structures and bridged them

(Fig. 12.4b). This approach has potential for engineering vascularized tissues that are capable of supplying sufficient oxygen and nutrients to entire tissue constructs.

12.6 Conclusion

This chapter has summarized approaches for modulating cell adhesion on a culture surface using external stimuli, such as thermal, electrical, photo, and magnetic fields. Electrochemical approaches for cell detachment are described in detail. These approaches have been used for several interesting purposes, including the detachment of specific cells at single-cell resolution under a microscope, the collection of spheroids, the fabrication of stacked multi-layered cell sheets, and engineering perfusable vascular structures in tissue constructs. These fundamental approaches will contribute to the further advancement of the fields of tissue engineering and regenerative medicine.

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