Precise tissue assembly using avidin-biotin binding system and optical tweezers

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Abstract

We have been investigating the feasibility of bottom-up tissue engineering, a new methodology to form tissues, by assembling single cells one by one using an avidin-biotin binding system (ABBS). In this report, we used optical tweezers in addition to ABBS to form small tissues where the cells alignment was precisely controlled. ABBS enabled very quick attachment of human hepatoma Hep G2 cells, and single cell manipulation by optical tweezers aligned the cells accurately. These results show that the combination of two technologies, ABBS and single cell manipulation, provides a new methodology for bottom-up tissue engineering.

Introduction

In tissue engineering, direct assembling of single cells to build tissues is one of the challenges. To achieve this final goal, it is necessary that a new method that can bind single cells in the moment in addition to single cell manipulation.

Avidin-biotin binding system (ABBS) is well known for its very strong binding affinity (K_d= 10^{-15} M), and used in many

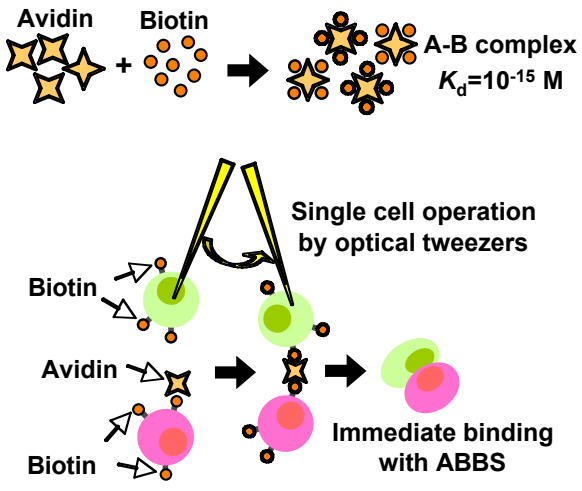

Figure 1 Schematic representation of the new method based on ABBS and optical tweezers An avidin protein consists of four subunits and each of them strongly binds one biotin molecule. In this study, we tested the feasibility of ABBS and optical tweezers to assemble cells precisely.

biochemical assays. We have been studying ABBS-dependent effective cell attachment onto a biodegradable polymer, poly-L-lactic acid, which has hydrophobic surfaces that often prevent efficient cell adhesion. ABBS is also very useful to trap cells not only onto two-dimensional surfaces but also complicated highly porous three-dimensional (3D) surfaces (Kojima *et al*). In these experiment, cells were likely to be attached very quickly onto the 3D biodegradable polymer scaffolds, because we inoculated the cells to the scaffolds by continuous shaking. We therefore expected that such very quick cell-to-cell attachment based on ABSS would be a key technology to provide a feasible method for bottom-up tissue engineering.

In this report, we demonstrate the feasibility of a new methodology that enables us to organize tissues with precise alignment of cells by using of both ABBS and optical tweezers.

Materials and Methods

Avidinylation and biotinylation of cells

Hep G2 cells, a human hepatoma cell line, were used as a model cells in all experiments. Hep G2 cells were biotinylated with commercially available reagent: EZ-Link Sulfo-NHS-LC-Biotin (Pierce). An aliquot of biotinylated-cells was then suspended into an avidin dissolved PBS solution to prepare avidinylated cells. Avidinylated and biotinylated cells were labeled with fluorescence dyes PKH67 (green) and PKH26 (red), respectively to distinguish with each other.

Optical cell trapping system

We used an optical tweezers system consisting of a microscope (Olympus) and a laser unit (Sigma Koki). This system was able to simultaneously control two laser tweezers to manipulate two single cells under the microscope observation.

Confocal microscopy

Assembled cell-based tissues were observed by a confocal microscope (Olympus).

Results and Discussion

We prepared two types of cells, one was an avidinylated Hep G2 cell and the other was a biotinylated Hep G2 cell. They were stained with green or red fluorescent dyes, respectively (**Figure 1**). First, we mixed these cells in a non-tissue culture treated plate and gently shook it for several seconds. The cells were immediately organized into aggregates due to the strong cell-to-cell bindings mediated by ABBS. Under confocal laser scanning microscopic observation, green-cells and red-cells were arranged like a checkered pattern (**Figure 2**). These results clearly show that ABBS can aggregate cells in very short time, almost immediately after the contact.

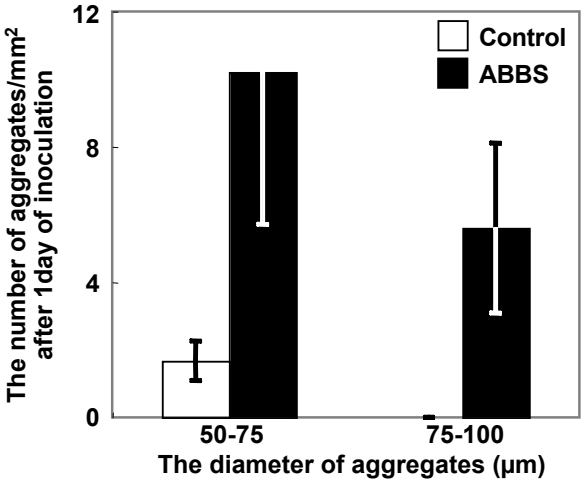

Figure 3 Cell aggregation with ABBS Avidinylated and biotinylated cells were mixed and cultured for 1 day with shaking. After the culture, we measured the diameter of aggregates. The number of aggregates (50-75 and 75-100 μm) were represented in the graph. Open bar: untreated cells. Closed bar: avidinylated and biotinylated cells. Data represent mean values ± S.D. of at least triplicate unit fields.

Second, to evaluate ABBS dependent development of aggregates, we continued the shaking culture for 24 hours. We categorized the aggregates with their diameter (50-75 or 75-100 μm) and counted the number in each group (**Figure 3**). Obviously, ABBS enhanced the development of aggregates when compared with control experiment without ABBS. We therefore concluded that ABBS contributes to the growth of large aggregates by its strong affinity that can bind two cells in a very short contact

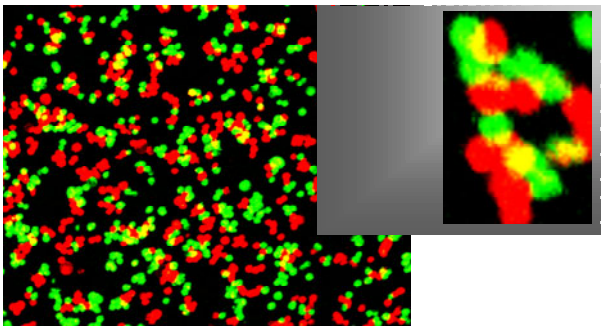

Figure 2 ABBS dependent cell attachment Avidinylated (red) and biotinylated (green) cells were mixed and shook together for several seconds. Two single cells that came into contact immediately formed binding. Yellow indicates the area in which red and green cells were attached

time under such continuous shaking conditions

Finally, we tried to assemble the cells more precisely using optical tweezers. We were able to control a single cell with the optical tweezers and to attach an avidinylated- and a biotinylated-cell immediately after the contact. After repeating such manipulations, chain-like tissues consisted of red- and green-cells were formed successfully (**Figure 4**). Although at present these tissues were very small (up to 20 cells), further assembling of these small tissues may lead to the formation of much larger tissues having a precisely-controlled microstructure.

ABBS has many advantages in addition to the quick cell-to-cell attachment as demonstrated in this study. The most important one is that ABBS is active under low temperature (4 ˚C). Such low temperature is likely to be helpful in organizing much larger tissues because cellular metabolic capacities are lowered, so that we can keep the cells alive without introducing flow of culture medium during the organization. Therefore, further proceeding of cell manipulation will allow the on demand building of tissues.

References

Kojima, N., Matsuo, T. and Sakai, Y. Rapid hepatic cell attachment onto biodegradable polymer surfaces without toxicity using an avidin-biotin binding system. *Biomaterials*, **27**, 4904-4910, (2006)

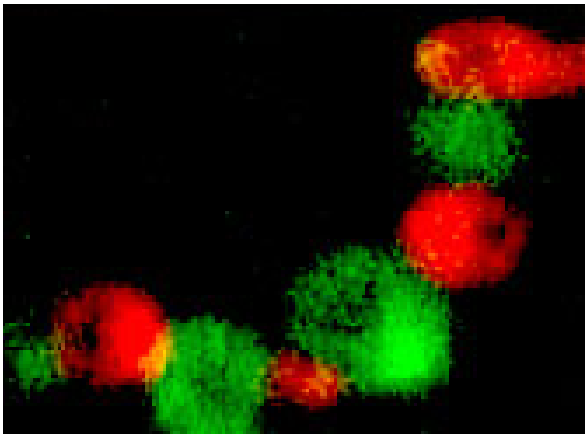

Figure 4 Chain-like tissue with ABBS Avidinylated (red) cells and biotinylated (green) cells were attached with optical tweezers one by one.