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ScienceDirect
Biomaterials 28 (2007) 1820–1829
Biomaterials
www.elsevier.com/locate/biomaterials

Selective control of liver and kidney cells migration during organotypic cocultures inside fibronectin-coated rectangular silicone microchannels

Eric Leclerc[a,*], Régis Baudoin[a], Anne Corlu[b], Laurent Griscom[c], Jean Luc Duval[a], Cécile Legallais[a]

[a]CNRS-UMR 6600, Laboratoire de biomécanique et génie biomédical, Université de Technologie de Compiègne, BP 20529, 60205 Compiègne Cedex, France
[b]Inserm-U522, Hôpital Pontchaillou, IFR 140, Université de Rennes I, 35033 Rennes, France
[c]CNRS-SATIE/BIOMIS, Ecole Normale Supérieure de Cachan, Campus de Ker Lann, Bruz, France

Received 24 October 2006; accepted 29 November 2006
Available online 18 December 2006

Abstract

In this work, the behaviors of embryonic liver and kidney explants were studied inside rectangular polydimethylsiloxane (PDMS) microchannels. The organs were cultured under monoculture and coculture conditions on PDMS coated with or without fibronectin. The results demonstrated that the migration of cells from both organs is dependent on culture conditions and thus can be selectively controlled. In liver monocultures without fibronectin, cell migration in the microchannels resulted in the formation of a dense 3D tissue. Fibronectin reduced liver cell migration and enhanced the emergence of cells demonstrating typical hepatocyte phenotypes at the vicinity of the explant. The migration rate in liver–liver cocultures, with and without fibronectin, was roughly twice the rate of cells under monoculture conditions. In cocultures, both livers merged to form a large tissue in which the two initial organs could not be identified. In kidney monocultures, with and without fibronectin, we did not observe any migration inside the microchannels. Contrary to liver cells, kidney cell migration was triggered when both fibronectin coating and coculture with liver or another kidney explant were used. The migration was more largely observed in coculture with liver when compared to kidney–kidney cocultures. In the case of liver–kidney coculture with fibronectin, the progression of the kidney cells inside the microchannels appears as a displacement of the entire kidney explant in the direction of the liver. The liver cells did not move in those cases. After contact, we observed a complete merging of both liver and kidney explants. In contrast, for liver–kidney cocultures without fibronectin, only the liver moved toward the kidney.
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Keywords: Rectangular microchannels; PDMS; Organotypic cultures; Liver; Kidney; Tissue engineering

1. Introduction

Tissue engineering is one of the promising fields that may lead to in vitro tissue and organ reconstruction ready for implantation [1–3]. For that purpose, a tissue scaffold is required as a physical framework in order to provide an initial cellular support so that the cells reach population with an appropriate density and functionality. Because of progress in microfabrication techniques, microchannels can be successfully manufactured in biocompatible and biodegradable polymers such as polydimethylsiloxane (PDMS), PLLA, PCL [4–8]. The microchannel scaffolds can be used to physically orient cell migration and/or proliferation and/or differentiation in order to promote the functional tissue replacements [9–13].

For in vitro liver reconstruction, cultivation of tissue in conditions comparable to that of the in vivo tissue is an important task to achieve physiologically significant functions for engineering the implantable tissue equivalents [14–16]. For that purpose, liver cells cocultures (hepatocytes with non-parenchymal cell [17,18] and hepatocytes with biliary cells [19]) have been developed, and these models have demonstrated that cell–cell interactions preserved hepatocytes, functionality [20]. Even if various studies are done on an artificial 3D matrix [21,22] and in coculture [23,24], the reconstructed physiology does not completely mimic in vivo 3D tissue.

*Corresponding author. Tel.: +33 3 44 23 79 43; fax: +33 3 44 23 79 42.
E-mail address: eric.leclerc@utc.fr (E. Leclerc).

0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.11.044

An alternative method of culture that allows tissues to keep a 3D multicellular organization is to use organotypic cell cultures. The method consists in working with excised tissue samples from embryonic explants and adult liver slices [25–30]. Using the technique of embryonic organotypic culture, microchannels can be used to physically orient the liver cell migration and the liver expansion along the length direction of the microchannels. Previous works observing liver tissue behaviors inside 6 types of PDMS microchannel geometries have demonstrated that the liver cells can migrate inside microchannels (depth × width × length, 135 μm × 150 μm × 5 mm) with an average up to 72 μm/day velocity over 14 days of experiment [31].

During morphogenesis, specific migration of cells is reported to drive the formation of many organ systems [32]. Recent studies have shown that the cell guidance is mediated by a combination of chemical and mechanical effects [32–34]. In this frame, chemotactism mechanisms appear to trigger the tissue development [35,36]. Using liver and kidney embryo cocultures, soluble factors secreted by embryonic liver such as transferrin has been reported to control kidney differentiation. Those works have also shown that liver-mediated stimulation induced the development and the expansion of the kidney mesenchyme [37,38]. Also, the extracellular matrix interacts with cells and regulates cellular functions such as adhesion, migration, proliferation, differentiation, and morphogenesis [39]. Fibronectin coating is reported to enhance hepatocytes differentiation [40,41].

To achieve tissue ready for implantation, it is necessary to accelerate the liver tissue development (cell migration and differentiation) inside the scaffolds. In this work, thanks to microchannel geometries and surface biochemistry modifications by coating with fibronectin, we develop an organ-to-organ interaction model to control and accelerate the migration step and differentiation step of the liver. For that purpose, we used cocultures of liver and kidney explants.

2. Materials and methods

2.1. Tested material

To fabricate the microchannels we used polydimethylsiloxane (PDMS, sylgard 184), silicone as it is ideally suited to microfabrication. PDMS has been widely used in emerging biotechnology fields to construct microchannels and microstructures with sub-micron features. As PDMS is a transparent material, microsystems that are fabricated with PDMS allow real time analysis of the morphological views of the cells and are largely used in the microtechnological fields [42,43].

2.2. Microfabrication and preparation of the microchannels

The PDMS microchannels were fabricated through replica molding processes with a mold master containing the negative pattern of the microstructures, as shown by Fig. 1A [44,45]. The detailed process is presented in our previous works [26,31]. Flat PDMS control samples were also prepared with 0.25 mm^2 surface area. The dimensions of microchannels are 135 μm × 150 μm × 5 mm (in depth × width × length).

2.3. Explant preparation

Chick embryos, incubated at 38.5 °C for 7 days, were removed from the eggs and rinsed in sterile phosphate buffered saline (PBS). Under a stereo microscope, the livers and kidney were extracted using microsurgical instruments and placed on an agar support medium. Each organ was divided to give explants of about 0.25 mm^2 sections (500 μm diameter).

2.4. Culture conditions

The organs were placed in Petri dishes containing 12 ml of a semi-solid culture medium composed of 37% Dulbecco's medium (Invitrogen, Cergy Pontoise, France), 10% fetal calf serum (Invitrogen, Cergy Pontoise, France), 2% glutamine (Invitrogen, Cergy Pontoise, France), 50% Bactoagar (Difco) 1% in Gey's saline solution. All PDMS samples were autoclaved before cell cultures and certain samples were coated with fibronectin (at 10 μg/ml, with a total volume of 10 ml, for 1 h at room temperature). The explants were then covered with the PDMS microstructured materials, as well as the flat PDMS control samples, as shown in Fig. 1B. In all cocultures experiments, the organs were separated by 2 mm as shown in Fig. 1C. The Petri dishes were incubated for 7 days, 14 days at 37 °C in a 5% CO_2 incubator.

2.5. Experimental methodology

We defined four situations: non-treated PDMS flat samples, non-treated PDMS microchannel samples, treated with fibronectin flat samples, treated with fibronectin microchannel samples. We investigated 5 cases, repeated 6 times, for each situation (case 1: $n = 6$ liver, case 2: $n = 6$ kidney, case 3: $n = 6$ liver–liver, case 4: $n = 6$ kidney–kidney, and case 5: $n = 6$ liver–kidney). One set of tests resulted in a total of 120 samples ($6 \times 5 \times 4$). We performed two sets of tests (2 times 120). Our observations were based on daily inverted, bright field microscopy analysis. For that purpose, the samples were photographed periodically by conventional numerical camera. Analyzed data are presented in terms of mean values and standard deviation (SD).

3. Results

3.1. Effect of fibronectin coating on liver or kidney explant mono cultures

3.1.1. Effect of fibronectin on liver cells

On all samples coated with fibronectin, including microstructured and flat control samples, tests using liver explants demonstrated indistinguishable behaviors over 14 days of culture.

On fibronectin flat PDMS sample controls, 12 h after organ seeding, cells with shapes characteristic of hepatocytes appear in the area surrounding the explants (Fig. 2A). Cells with hepatocyte phenotype (morphology) were observed during 72–96 h for all tests and then disappeared (Fig. 2B). In contrast, no cells surrounding the explants were observed (data not shown) on non-treated flat PDMS samples. Finally, after 1 week of cultures, tests with and without fibronectin coating on the flat samples led to similar liver explant morphology (Figs. 2B and C).

On microchanneled samples coated with fibronectin, cells with hepatocyte morphology emerged from the liver

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