Chapter 14

Time-lapse technologies and 4D imaging of kidney development

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Abstract

Time-lapse imaging a technique of frequent imaging and following a course of a process. Because the development of the embryonic kidney can proceed *ex vivo* after dissection, it is possible to study the morphogenesis by culturing the kidney in the on-stage incubator of a microscope and follow the developmental process by imaging. Confocal microscopes and other three-dimensional imaging systems offer the possibility for tracking the development process in four dimensions – 3D and the time.

Key words: time-lapse imaging, kidney development, tissue culture

1 Introduction

Kidney development has been studied using time-lapse imaging already before digital cameras [1,2,3]. The digital cameras and the genetically modified mice have made the time-lapse techniques more available to study the morphogenesis in details. The GFP label on the cell membrane was introduced to study the branching of the ureteric bud [4]. Also patterning of the collecting duct system [5], behavior of cap mesenchymal cells [6] and self-organization after the dissociation of the kidney [7] have been studied using time-lapse imaging.

It is possible to apply cell segmentation and quantification in time-lapse images to detect individual cells and to follow their movement in the short time-range [8]. In this method, the embryonic kidney or kidney organoid is placed to grow in the restricted z-space making the explant grow in a flatten sheet of 70 µm thickness (Fig. 1). Because of the reduced thickness of

the tissue, less z-planes are needed for the 3D imaging. In this system, there is only a cover glass between the imaged object and the objective and this also improves the quality of the images and makes it possible to reduce the laser power, damaging the tissues less. The images can be taken with good resolution (voxel size 0.13x0.13x1.13µm) and with high frequency, at least every 5 minutes.

2 Materials

2.1. Fixed Z-direction culture

1. 6-well Greiner CELLSTAR® multiwell culture plates (M9062 Sigma)
2. 24 mm Transwell® with 0.4 µm Pore Polyester Membrane Insert (CLS 3450, Sigma)
3. 70 µm polystyrene beads (100263-10, Corpuscular)
4. Matrigel® (Corning) - optional
5. Histoacryl® glue (Braun Ref 1050052)
6. 24x24 mm cover glasses (cleaned according the protocol)
7. 1M HCl
8. Dulbecco's Modified Eagle's Medium (DMEM, D777 Sigma)
9. Fetal Bovine Serum (FBS, Gibco)
10. Penicillin and Streptomycin (P/S, Sigma)

3 Methods

3.1. Preparing the plate for imaging

1. Drill 20mm in diameter holes in the bottom of wells. (Fig.2, see **Note 1**)
2. Smoothen the rims of the holes by rubbing with a pumice.
3. Glue the cleaned coverslip on top of the hole using the Histoacryl® glue.
4. Before using rinse with ethanol, distilled water, and dry in a UV hood.

3.2. Cleaning of the cover glass

1. Heat cover slips in a loosely covered glass beaker in 1M HCl at 50-60 C for 4-16h.
2. Cool to room temperature
3. Rinse out 1M HCl with ddH2O
4. Fill container with ddH2O and sonicate in water bath for 30 mins
5. Repeat step 4
6. Repeat step 4
7. Fill container with 50% EtOH and 50% ddH2O and sonicate in water bath for 30 min
8. Fill container with 70% EtOH and 30% ddH2O and sonicate in water bath for 30 min
9. Fill container with 95% EtOH and 5% ddH2O and sonicate in water bath for 30 min
10. Fill container with 95% EtOH
11. Keep coverslips on Whatman filter paper in a box and keep for autoclave.

4 Methods

4.1. Fixed Z-Direction (FiZD) culture system

1. Dissect kidneys and keep in the culture medium (see **Note 2**)
2. Mix an aliquot of polystyrene beads in Matrigel (on ice) and make 6-7 drops of Matrigel on the lower side of Transwell insert (chilled on ice) (see **Note 3**)
3. Add the kidneys on the drop of Matrigel or on the lower side of the Transwell insert. In the latter case, add the polystyrene beads at the bottom of the well on the cover glass. (see **Note 4**)
4. Turn the insert around and transfer it into the well very gently.
5. Compress the kidney samples very slowly and gently until they are 70 µm in depth (control under the microscope).
6. When the samples are assembled, fix the inserts in the wells by melting the plastic at three positions with hot glass or metal needle.
7. Add about 2ml of culture medium (DMEM/20 % FBS/1 % P/S) to the well. Place to the incubator at 37 °C and 5 % CO_2.

4.2. Time-lapse imaging

1. Turn on the microscope (Zeiss LSM 780 confocal microscope) and the on-stage incubator. Wait until the incubator temperature and carbon dioxide levels have settled to 37 °C and 5 % CO_2.
2. Place the 6-well plate with the samples in the incubator.
3. Determine the position of the imaged samples and the imaging settings.
4. Determine the number and position of z-stacks.
5. Start the time-lapse imaging. Imaging set for every 5 to 20 minutes.
6. Save the files daily.

4.3. Image processing

1. Deconvolute the data with Huygens Professional (Scientific Volume Imaging).
2. Combine the image data from different files to include the whole imaging time.
3. Further enhancement of the image quality and the segmentation of the data can be done using Matlab®(Mathworks) based programs or Fiji [9].

5 Notes

1. Wear protective glasses and respiration filter because of small plastic shreds.

2. The culture method can be used also for kidney organoids and other tissues such as gonads [10]

3. Keep the plate also on ice to chill the glass bottom.

4. Matrigel is not necessary to use

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Figure 1. Embryonic mouse kidney cultured in the FiZD culture system for 12 days. Collecting ducts marked by Troma (red) and the glomeruli by Nephrin (green). Reproduced from (Saarela et al., 2017) with permission from the Development.

Figure 2. A modified 6-well plate with cover glass glued in the bottom of the wells. A Transwell® insert placed in well 2.