Construction of Nephron by Fusion of Adult Glomeruli to Ureteric Buds with Type V Collagen

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Abstract

Although tissue engineering of artificial organs such as skin or cartilage have been developed greatly, organs such as kidney or liver have not been completed yet. Human kidney consists of one million of nephrons which is the basic unit for the filtration of blood, excretion of urine, secretion of cytokine and re-absorption of electrolytes. Every nephron comprising glomerulus and tubule with complicated structure and full of blood capillaries can hardly be re-constructed by current techniques. In this study, we constructed nephrons by fusing adult glomeruli to ureteric buds with a newly dynamic scaffold-type V collagen fiber under microgravity. We firstly proved that type V collagen fiber can not only provide a dynamic scaffold for renal glomerular endothelial cells but also can induce other ECM (excellular matrix) such as type IV collagen and fibronectin which are the main compoments for glomerular basement membrane. By using type V collagen fiber, we challenged a new concept different from the known tissue engineering technique in which stable scaffold such as collagen I was generally used. In our experiment, adult mice glomeruli and embyonic ureteric buds could be fused to form a new nephron *in vitro* and blood capillaries could be induced around glomeruli when both developmental and aging tissues were co-cultured on type V collagen fiber under microgravity. We successfully constructed nephrons by fusing the adult glomeruli to the embryonic ureteric buds by using a dynamic scaffold. Such techniques may be useful to the application of regeneration of kidney in the future.

Introduction

Development of renal tissue requires various phases that induce embroynic mesenchyme changing into epithelium and verse versa. Such kind of phase change requires ECM mediator not only to provide cell and tissue with synergic extracellular environment but also to induce the migration and aggregation of cell and tissue to form phase changes. Our experimental results of culturing mice adult glomerular endothelial cells and embryonic kidney tissue on type V collagen proved that Type V collagen fiber is a suitable ECM mediator for such kind of phase changes. For adult cells, we found that type V collagen fiber could not only induce the migration of glomerular endothelial cells dynamically, but also enhance the cell-cell interaction in the super structure of ECM. In addition, type V collagen was also found to turn over the dynamic phase into stable phase of ECM structure and therefore it could provide a transitional environment for cells. For embryonic kidney, we found that type V collagen fiber could induce mesenchymal cells to form vascular morphogenesis. Type V collagen was found to incorporate into the branching tip of ureteric buds and then distributed from

whole area of ureteric buds to the leading portion of glomeruli during metanephors development. This fact suggests that type V collagen played a guiding role between the interface of mesenchume and epithelial tissue. Moreover, the fact that type V collagen fiber existing at immature glomeruli while type IV collagen at mature glomeruli suggests that phase change of ECM occurred during kidney development. All these facts proved that type V collagen functions the tissue plasticity and the dynamic tissue-tissue interaction for renal tissue morphogenesis. The objective of this study is to utilize type V collagen as the leading ECM to construct kidney tissue, which comprises inducing the interactions among glomerular microvascular endothelial cells, smooth muscle cells and tubular epithelial cells, and formation of kidney tissue from developing metanephon in vitro.

Materilas and Methods

Glomeruli were isolated from kidney of adult iCR mice of 6 weeks, and ureteric buds of metanephros were isolated from fetal iCR mice of E11.5. Type V collagen molecule was extracted from porcine cornea by pepsin treatment under acidity condition. Type V collagen fiber was constituted by neutralizing type V collagen molecule with urea and NaCl. The media used for tissue culture was EN/EP (1:1) media (EN medium per liter: Ham F-12, 5.3g; DMEM, 5g; $NaHCO_3$, 1.9g, ITS premix, 10 ml; EGF, 500ng; FBS, 10%. EP medium per liter: RPMI, 10.4g; heparin, 50mg, β ECGF, 10 μ g, FBS, 10%). All the tissue culture was performed under 37^{o}C with 5% CO_2 and 95% humidity. For the fusion experiment of adult glomeruli and embryonic metanephos, Type V collagen fiber was used as ECM and commercial type I collagen was used as control scaffold for all experiments. Adult glomeruli was firstly cultured on mebiol-gel mixed with type V collagen fiber for 3 days and then added to embryonic metanephros which were treated with 0.1% of mixed collagenase of I, IV and XI in PBS for 2 minutes at room temperature and washed with PBS right before fusion experiment. EN/EP media were then added to the tissue culture and the mixture of adult glomeruli and embryonic metanephros were cultured for four more days. After the tissue culture, samples were subjected to contrast microscopy for the observation of the morphological change of glomeruli and metanephros. Immunohistochemistry was also performed on frozen sections of the cultured tissue to identify the location of type V collagen fiber. In order to distinguish the tubule tissue from glomeruli tissue, Dolichos biflorus agglutinin was used to stain ureteric buds and Anti-type V collagen antibody was used to detect the localization of type V collagen fiber in the cultured tissue.

Results

Comparison between the available method for tissue culture of metanephros and our method are shown in Figure 1. The existing method resides on the tissue culture of ureteric buds of metanephros to extend the nephorn tubules by forming the epithelialized metanephron (Fig. 1a) , while our methods resides on the formation of glomeruli and microvascular, the most difficult tissue

morphogenesis, in kidney. In addition of the glomeruli and microvascular formation, our method also resides on the tissue connection between tubules and glomeruli (Fig. 1b).

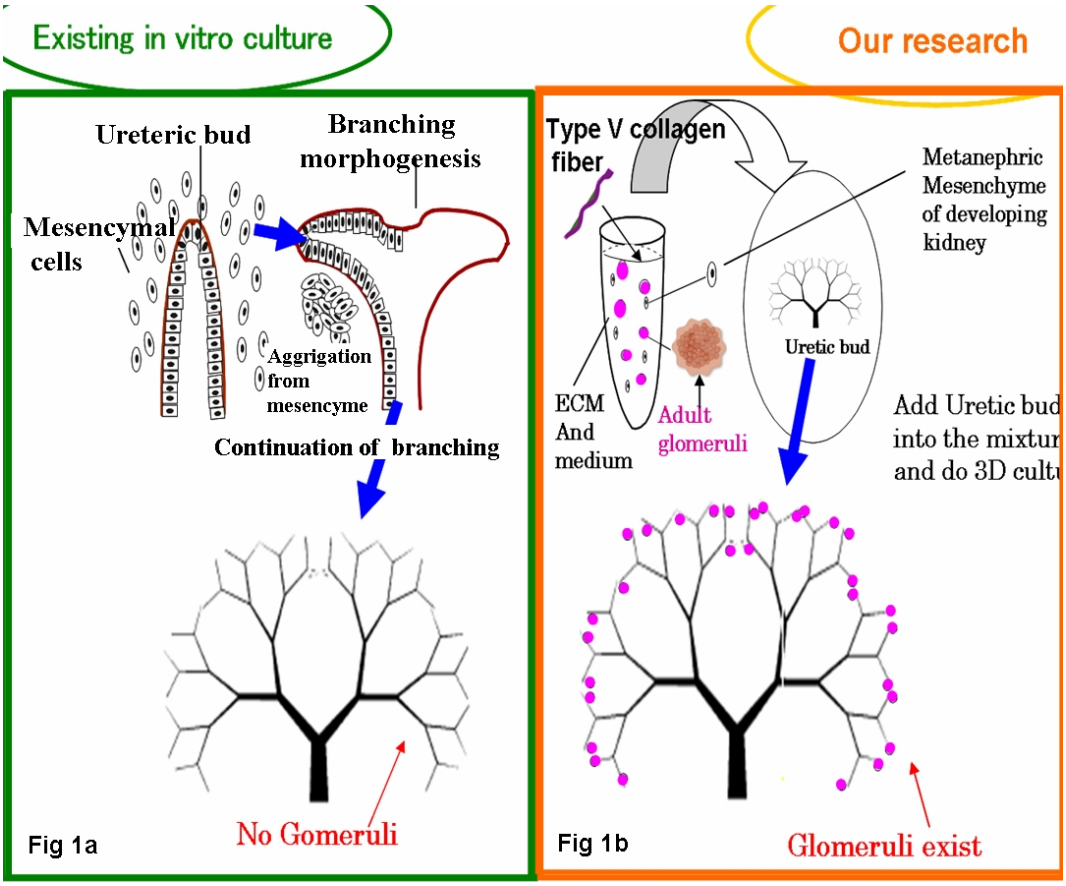

Figure 1 Outline of this research: Existing method (1a) and our method (1b)
Figure 1a resides on the tissue culture of ureteric buds of metanephros to extend nephorn tubules. Figure 1b resides on the formation of glomeruli and microvascular and the connection between tubules and glomeruli.

The cultured tissue was subjected to contrast microscopy and fluorescent microscopy, respectively, and the results were shown in Figure 2a (magnified: 200), Figure 2b (magnified: 400) and Figure 2c (magnified: 630). Contrast microscopy indicated that branching of ureteric buds of embryonic metanephors was successfully induced and adult glomeruli (green dotted circle in Fig. 2a) fused to the tip of ureteric buds (pink dotted circle in Fig. 2b) on type V collagen fiber after 7day culture. Fluorescent microscopy of cultured tissue shows that ureteric buds were stained by dolichos biflorus agglutinin in green color and type V collagen fiber was stained by anti-collagen V antibody in red color. It is interesting to find that type V collagen not only surrounded the adult glomeruli (white dotted circle in Fig. 2c) but also surrounded around the ureteric buds. indicated that tip of branched ureteric bud was aggregated (blue dotted circle in Fig. 2c). It is obvious that type V

collagen plays a fusion bridge role between adult glomeruli and ureteric buds.

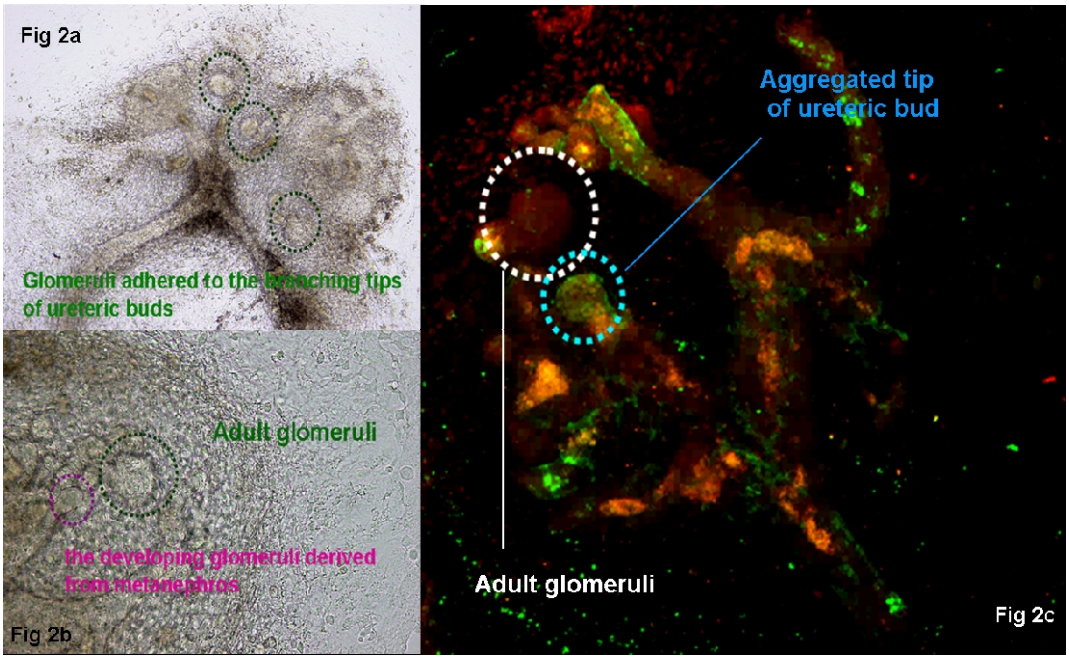

Figure 2 Microscopy of the cultured tissue of adult glomeruli with E11.5 embryonic metanephros after 1 week culture.

Figure 2a shows the phase contrast microscopy of the tissue culture of adult glomeruli and embryonic metanephros; Figure 2b is the enlargement of Figure 2a. Green dotted circle: adult glomeruli; pink dotted circle: embryonic gloeruli.

Figure 2c shows the fluorescent microscopy of the tissue culture of adult glomeruli and embryonic metanephos. White dotted circle: adult glomeruli, blue dotted circle: tip of embryonic ureteric buds. Green color: ureteric buds were stained by dolichos biflorus agglutinin; red color: type V collagen fiber.

Figure 3 shows the location relationship between type V collagen fiber, adult glomeruli and embryonic ureteric buds after 7 day tissue culture. Type V collagen fiber (stained in red color) was distributed homogeneously in the media at the initial culture stage (data not shown). It is surprising to find that type V collagen fiber formed as a big bundle-like structure after 7 day culture with tissue, and this bundle surrounded the embryonic ureteric buds (stained in green color) and extended to the adult glomeruli (blue dotted circle). It seems that type V collagen bundle (stained in red color) dragged the adult glomeruli to the tip of embryonic ureteric buds (yellow dotted circle) and made a connection between both adult glomeruli and ureteric buds.

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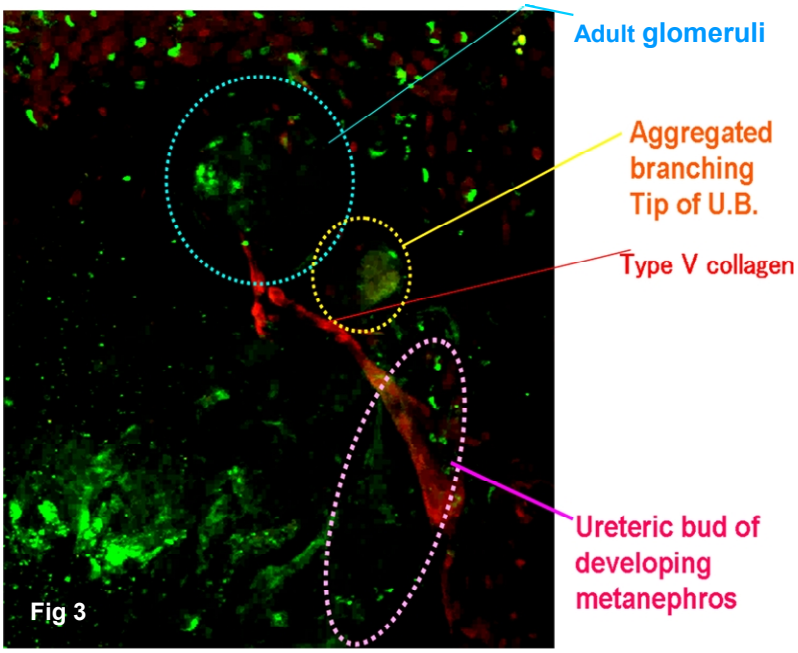

Figure 3 Immunochemistry microscope of the cultured tissue.
Type V collagen fiber plays a bridge role between adult glomeruli and embryonic kidney.
Green shows the epithelial marker of sinaptopodin and red shows the marker of type V collagen fiber.

Discussion

At adult kidney, collagen type V induces collagenofibrotic glomerulopathy (ref 1). Functions of type V collagen are not only to connect type IV collagen with type I collagen fibril, but also to protect the parenchyma from excess type I collagen deposition produced by stellate cells under pathological conditions (ref.2).At the developmental stage of kidney, type V collagen transiently appeared, but we have not knew what type V collagen doing.

On the basis of above results, we hypothesized a model of the fusion between adult glomeruli and embryonic ureteric buds in Figure 4. At the pre-treatment stage, cells of adult glomeruli outgrew from the glomeruli and invaded to the mebiol-gel. The invading cells secreted matrix protein to surround the surface of glomeruli, simultaneously the secreted matrix protein took into type V collagen fiber mixed in the mebiol-gel and coated glomeruli with type V collagen fiber richly. Since we had proved previously that type V collagen fiber could be easily taken into cultured cell or tissue and enhanced the dynamism of cells and also proved that type V collagen could enhance the dynamism of ECM due to its rapid digestion by MT1-MMP from kidney cells and structural smooth fiber-fiber interaction (ref.3), we hypothesized that type V collagen fiber was taken into the cultured tissue and was recognized by embryonic mesenchymal cells which then were connected

and sourrounded by adult glomeruli richly coated with type V collagen fiber (Fig. 4). The immunohistochemistry results revealed that type V collagen fiber formed a bundle structure surrounding of ureteric bud (Figure 4b). The bundle collagen further extended to the glomeruli so as to drag ECM-enriched glomeruli close to the tip of ureteric buds; finally it caused the fusion of glomeruli to ureteric buds. In conclusion, type V collagen fiber plays a bridge role to connect the adult glomeruli and embryonic ureteric buds and form a complete nephron.

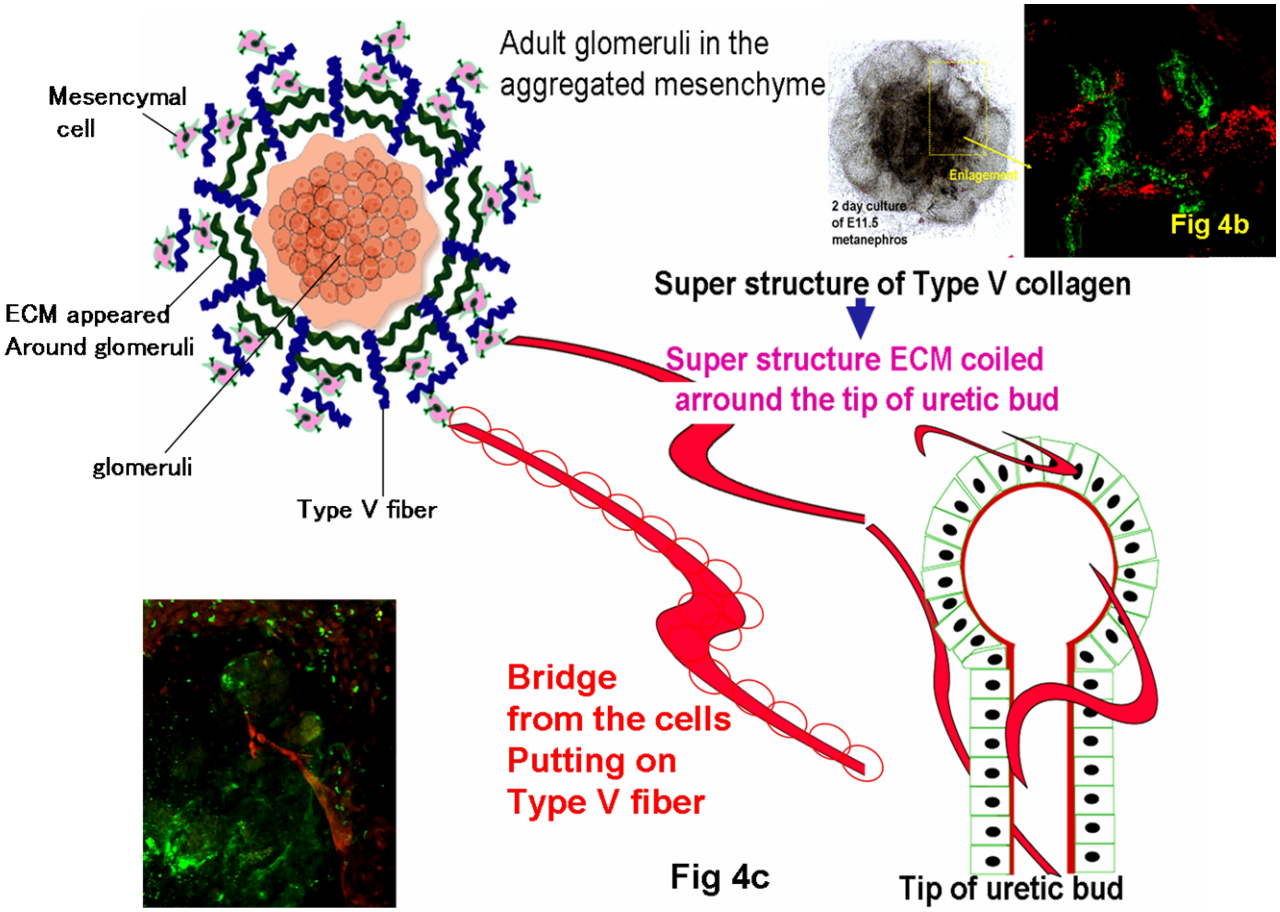

Figure 4 Hypothetical fusion model between adult glomeruli and embryonic kidney

References

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