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Original Papers

Generation of Tubular Superstructures by Piling of Renal Stem/Progenitor Cells

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ABSTRACT

The aim of this study was to assess cell biological processes involved in kidney regeneration. Particular interest was focused on the development of renal stem/progenitor cells into structured tubules. To investigate this process, the availability of a powerful cell culturing system is important. One prerequisite is that embryonic tissue can be maintained for several weeks to observe the progression of tubule development under physiological and under pathological conditions. Additionally, a significant amount of tissue must be harvested to analyze the cellular processes involved in the formation of tubules. In the present experiments, it was demonstrated for the first time that the spatial development of tubules could be modulated by piling stem/progenitor cells derived from neonatal rabbit kidney. Isolated embryonic tissue was mounted inside a specific holder and covered by layers of polyester fleece to create an artificial interstitium. The tissue was cultured in a perfusion container for 13 days in a chemically defined Iscove's modified Dulbecco's medium containing aldosterone (1×10^{-7} M) as a tubulogenic factor. The spatial development of tubules was registered on whole-mount specimens and on cryosections labeled with lectins or tissue-specific antibodies. The experiments revealed that culturing renal stem/progenitor cells in one layer resulted in the development of numerous differentiated tubules lining one row. Increasing the layers of renal stem/progenitor cells by piling resulted in the formation of multiple tubules orientated in parallel rows. Consequently, this technique made it possible to pile renal stem/progenitor cells like bricks and experimentally extend the spatial environment for tubular growth. This novel technique is an important step toward the generation of renal superstructures urgently needed for biomedical research and kidney regeneration.

INTRODUCTION

THE USE OF STEM CELLS in renal injury and repair is the subject of intense clinical and biomedical research.[1–4] However, the future success of this therapeutic strategy depends on the adequate integration of stem cells in the diseased organ.[5–7] Additionally, it depends on the capabilities of stem cells to regenerate functional parenchyma within a pathophysiological environment.[8–10] Besides these unsolved problems, an anatomically complicated organ, such as the kidney, which comprises multiple cell types and exhibits a sophisticated three-dimensional structure, makes it difficult to analyze stem cell development into functional parenchyma. These issues make it difficult to uncover ways to therapeutically influence cell biological mechanisms involved in a regeneration process.[11]

Tubular development within the kidney occurs in a spatial environment.[12,13] To simulate this environment and to investigate the formation of tubules, advanced culture techniques are required.[14,15] A presumption is that renal stem/progenitor cells can develop in an appropriate spatial environment. Additionally, developing tissue will need to

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be maintained over prolonged periods of time to investigate the progression of tubule formation. However, to exclude uncontrollable influences, the coating of embryonic renal tissue with extracellular matrix compounds should be avoided.[16,17] To maintain a controlled provision of nutrition and respiratory gases, the perfusion culture should be maintained with fresh and chemically defined medium.

In previous papers, we demonstrated that an environment for the spatial development of tubules derived from renal stem/progenitor cells can be created at the interphase of an artificial interstitium made of polyester fleeces.[18–21] In the present experiments, we showed for the first time that culturing tissue at the interphase of an artificial interstitium can be used to extend the spatial environment for tubular growth by piling and paving tissue within a perfusion culture container. New information concerning the feasibility for the spatial formation of tubules was obtained in these experiments. Thus, piling of renal stem/progenitor cells appears to be an appropriate strategy to extend systematically the spatial formation of tubules under controlled culture conditions.

MATERIALS AND METHODS

Isolation of embryonic explants containing renal stem/progenitor cells

One-day-old New Zealand rabbits were anesthetized with ether and killed by cervical dislocation. Both kidneys were removed immediately. Each kidney was dissected into two parts. By stripping off the capsula fibrosa with fine forceps, a thin embryonic tissue layer containing numerous collecting duct ampullae, S-shaped bodies, and nephrogenic mesenchyme was harvested (Fig. 1A).[22] The isolated embryonic renal tissue was placed between two punched-out layers of polyester fleece (Walraf, Grevenbroich, Germany) measuring 5 mm in diameter. This arrangement resulted in a sandwich-like configuration (Fig. 1B, C) with the freshly isolated embryonic tissue in the center and the two layers of polyester fleece covering the outer sides. These basic sandwich setups could be stapled like bricks. For the first time, piling several sandwiches in this manner enabled us to generate renal tubules stepwise in an extended spatial environment.

Basic sandwich setup in a perfusion culture container

For piling experiments, the basic sandwich setup must be held in a specific position during culturing so that damage of the growing tissue is prevented. Thus, a base ring of a Minusheet® tissue holder with 13 mm inner diameter was transferred to a perfusion culture container with horizontal flow characteristics (Minucells and Minutissue, Bad Abbach, Germany).[20,21] A polyester fleece measuring 13 mm in diameter was mounted into the tissue holder (Fig. 1D). A basic sandwich setup (Fig. 1B, C) containing renal stem/progenitor cells was inserted. Finally, the cover of a 13 mm polyester fleece was placed on top of the sandwich. After closing the lid of the perfusion culture container, the complete construction was fixed in an exact position for the entire period of perfusion culture.

Piling up basic sandwich setups

To investigate the spatial formation of generated tubules, piling of renal stem/progenitor cells was performed. For these experiments, the basic sandwich setup containing renal stem/progenitor cells was modified. First, an isolated embryonic renal tissue was placed between two punched-out layers of polyester fleece measuring 5 mm in diameter (Fig. 1B, C). Then, a layer of isolated embryonic renal tissue was mounted on top and covered by a fleece measuring 5 mm in diameter. This double sandwich setup, which included two layers of freshly isolated embryonic renal tissue, was used in a tissue holder as described previously. For paving, the basic sandwich setup including renal stem/progenitor cells was placed beside each other within a tissue holder.

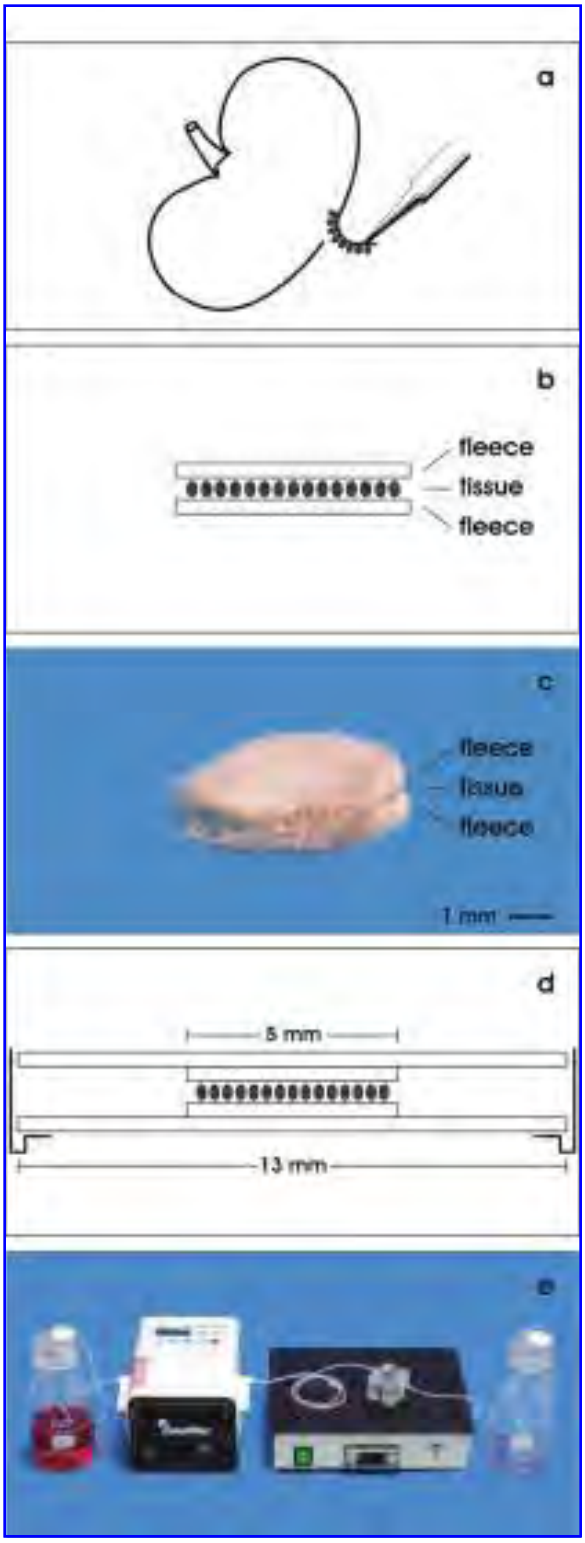
Perfusion culture to generate renal tubules

Perfusion culture was performed as described (Fig. 1E).[21,23] To generate renal tubules, chemically defined IMDM (Iscove's modified Dulbecco's medium including Phenol red; GIBCO, Karlsruhe, Germany) was used. Up to 50 mM HEPES (GIBCO) was added to the medium to maintain a constant pH of 7.4 under atmospheric air containing 0.3% carbon dioxide. To promote tubulogenic development, aldosterone (1×10^{-7} M; Fluka, Taufkirchen, Germany) was added to the culture medium. An antibiotic–antimycotic cocktail (1%; GIBCO) was present in all culture media. Fresh medium was continuously perfused for 13 days at a rate of 1 mL/h with an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany). To maintain a constant temperature of 37°C, the culture container was placed on a thermoplate (Medax, Kiel, Germany) and covered with a transparent lid.

Lectin and antibody labeling

Developed tubules were analyzed as whole-mount specimens and as cryosections in longitudinal or cross-sectional view. Cryosections of 20 μm thickness were fixed in ice-cold ethanol. After washing with phosphate-buffered saline (PBS), the specimens were blocked with PBS containing 1% bovine serum albumin (BSA) and 10% horse serum for 30 min. For lectin labeling, the samples were exposed to fluorescein isothiocyanate (FITC)–conjugated lectin diluted 1:2000 in blocking solution for 45 min as described.[23] For antibody labeling, monoclonal antibodies were applied undiluted or diluted as primary antibodies for 1 h (Table 1). After a washing step with 1% BSA in PBS, the specimens were incubated for 45 min with donkey anti-mouse IgG FITC, donkey anti-goat IgG FITC, or goat anti-rat IgG rhodamine (Jackson Immunoresearch Laboratories, West Grove, PA) diluted 1:50 in PBS containing 1% BSA. Fol-

lowing several washes in PBS, the sections were embedded with Slow Fade Light Antifade Kit (Molecular Probes, Eugene, OR) and then analyzed using an Axioskop 2 plus microscope (Zeiss, Oberkochen, Germany). Fluorescence images were taken with a digital camera at a standard exposure time of 1.3 s and thereafter processed with CorelDRAW 11 (Corel, Otawa, Canada).



Scoring

Tubular structures exhibited polarized cells, a visible lumen, and a basal lamina. According to the profile of Soybean Agglutinin (SBA) labeling, we used a specific score for evaluation of tubule development as described. The presence of tubules, their number, their length, intensity of label, lumen formation, and the existence of a basal lamina were registered. Each criterion was scored with one point, resulting in a possible maximum of six points.

Morphometry

To determine the amount of developed tubules, SBA-labeled whole-mount specimens were used. To register the number of generated tubules, WCIF ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD), a morphometric program, was used (Fig. 2B). Independent of its length, each SBA-labeled tubule within a microscopic opening of $620 \times 930\,\mu m$ was registered.

Amount of cultured constructs

A total of 97 embryonic tissues were isolated and maintained in culture for the present study. All the experiments were performed at least in triplicate. The data provided in the text are the mean of at least three independent experiments. All experiments were in accordance with the Animal Ethics Committee, University of Regensburg, Regensburg, Germany.

RESULTS

The aim of the present experiments was to generate tubules derived from renal stem/progenitor cells in an extended spatial arrangement under controlled *in vitro* conditions. We used a basic sandwich setup, containing renal stem/progenitor cells, to pile them like bricks in a perfusion culture container at the interphase of an artificial interstitium. These experiments should reveal the technical feasibility of extending step by step the spatial environment for the formation of tubules.

FIG. 1. Illustration of the culturing setup to generate renal tubules at the interphase of an artificial interstitium. Isolated embryonic renal tissue derived from the outer cortex of neonatal rabbit (**A**) was placed between two punched-out layers of polyester fleece measuring 5 mm in diameter (**B**). This basic sandwich setup (**B, C**) was placed in a Minusheet holder covered at the base and top with a polyester fleece measuring 13 mm in diameter (**D**). The assembly was used in a perfusion culture container with horizontal flow. Medium was continuously perfused for 13 days at a rate of 1 mL/h with an IPC N8 peristaltic pump (**E**). To maintain a constant temperature of 37°C, the culture container was placed on a thermoplate and covered with a transparent lid. Color images available online at www.liebertpub.com/ten.

Table 1. Histochemical Markers Demonstrating Differentiation in Generated Tubules and on the Collecting Duct (CD) Ampulla (Amp) within the Embryonic Zone of Neonatal Rabbit Kidney

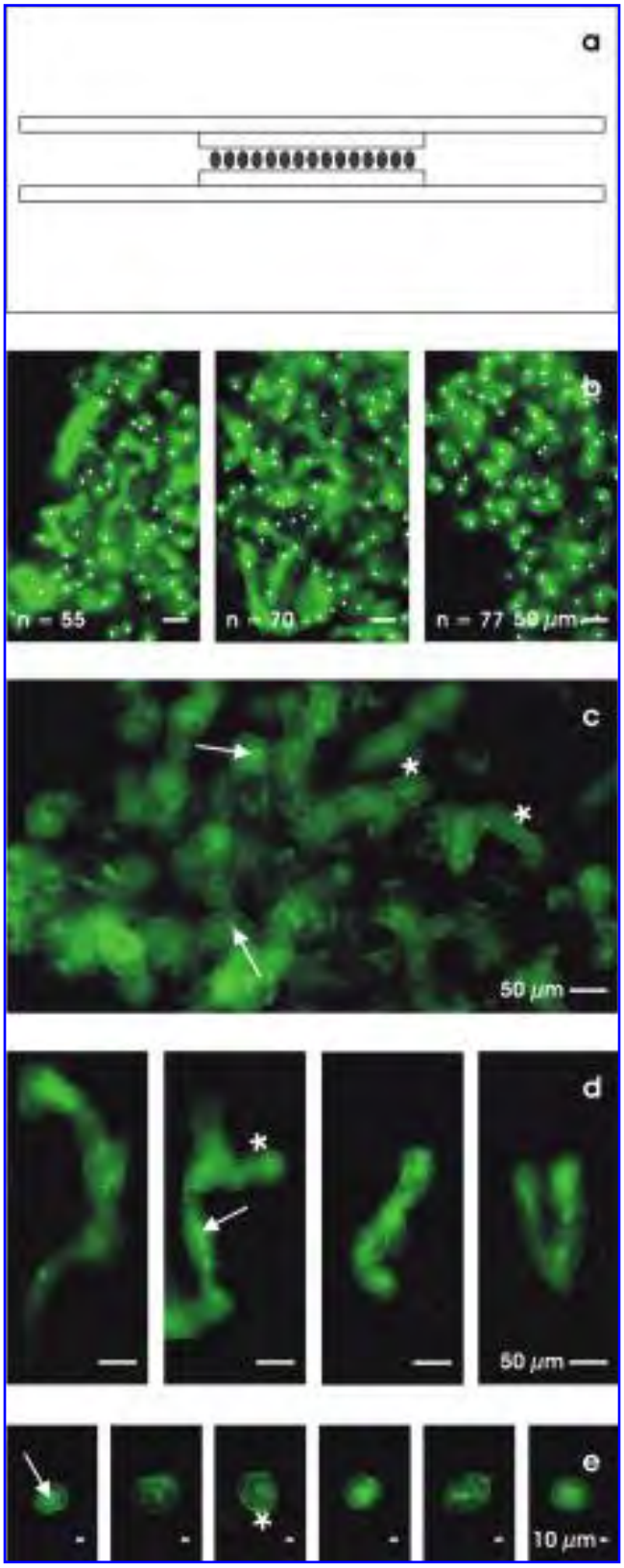
Marker	*Distributor*	*Dilution*	*Label on tubules*	*CD Amp*
Dolichos Biflorus Agglutinin (DBA)	Vector	1:2000	+	−
Griffonia Simplicifolia Lectin (GSL)	Vector	1:1000	+	−
Soybean Agglutinin (SBA)	Vector	1:2000	+	−
Ulex Europaeus Agglutinin I (UEA I)	Vector	1:1000	+	−
Vicia Villosa Lectin (VVL)	Vector	1:500	+	−
Wheat Germ Agglutinin (WGA)	Vector	1:500	+	+
Occludin	Zymed	Undiluted	+	+
E-cadherin	Zymed	1:10	+	+
Desmoplakin I/II	Santa Cruz	Undiluted	+	−
Na/K-ATPase	Developmental Studies Hybridoma Bank, Iowa	Undiluted	+	−
COX 2	Santa Cruz	1:5	+	−
Calbindin-D-28K	Sigma	1:50	+	−
Renal P_{CD3}	Mab/own lab	Undiluted	+	−
Renal P_{CD6}	Mab/own lab	Undiluted	+	−
Cytokeratin 19	Progen	Undiluted	+	+
TROMA-I	Developmental Studies Hybridoma Bank, Iowa	Undiluted	+	+
TROMA-III cytokeratin 19	Developmental Studies Hybridoma Bank, Iowa	Undiluted	+	+
Fibrillin-1	Santa Cruz	1:10	+	−
Laminin γ1	Dr. L. Sorokin	Undiluted	+	+
Transglutaminase II	NeoMarkers	1:5	+	+
Ezrin	Santa Cruz	Undiluted	+	−
Agrin	Developmental Studies Hybridoma Bank, Iowa	1:20	+	+

Generation of a single row of tubules

After stripping off the capsula fibrosa from the neonatal rabbit kidney, a thin tissue layer containing numerous stem/progenitor cells was isolated (Fig. 1A). In the first set of culturing experiments, the isolated embryonic tissue was placed between two layers of polyester fleece (Fig. 1B, C; Fig. 2A). The area for tubule formation was 5 mm in diameter, and the spatial environment was up to 250 μm in height. The interphase between the fleece layers produced an artificial interstitium with an optimal microenvironment for the development of tubules during a 13-day culture period. Labeling whole-mount specimens with SBA revealed numerous and densely packed tubules (Fig. 2B). To obtain information about the number of developed tubules, each SBA-labeled tubule was registered in a microscopic opening of 620×930 μm, independent of its length. We detected between 55 and 77 tubules within a defined area of the fleece.

Fluorescence microscopy of whole-mount specimens cultured for 13 days in chemically defined IMDM further demonstrated that SBA-labeled tubules were found beside unlabeled tissue. Most of the generated SBA-labeled tubules were detected in an oblique perspective, which made a proper morphological analysis difficult (Fig. 2C). However, part of the SBA-labeled tubules could be observed in longitudinal view and represented lengths ranging between 200 and 450 μm (Fig. 2D). Part of generated tubules could be analyzed in the vertical view (Fig. 2E). Both the longitudinal view and vertical view exhibited a smooth basal lamina, lining epithelial cells and a clearly visible lumen on SBA-labeled tubules. Placing basic sandwich setups side by side revealed that within a diameter of 10 mm numerous developed tubules with same features of differentiation were found (not shown).

For further cell biological characterization, cryosectioning following SBA labeling of cultured tubules derived from a single layer of embryonic tissue was performed (Fig. 3). Low magnification revealed numerous developed tubules embedded in loose connective tissue at the interphase of an artificial interstitium after a 2-week culture period (Fig. 3B). SBA staining of longitudinal (Fig. 3C) and cross-sectioned (Fig. 3D) specimens demonstrated intensively labeled tubules exhibiting a basal lamina, lining epithelial cells, and a visible lumen under higher microscopic magnification. Further experiments demonstrated that besides SBA, numerous other lectins and antibodies reacted on generated tubules (Table 1). The list of markers comprised lectins such as Dolichos Biflorus Agglutinin (DBA), Griffonia Simpli-



cifolia Lectin (GSL), Ulex Europaeus Agglutinin I (UEA I), Vicia Villosa Lectin (VVL), and Wheat Germ Agglutinin (WGA), or antibodies against cellular or extracellular proteins, such as occludin, E-cadherin, desmoplakin I/II, Na/K-ATPase, COX 2, calbindin-D-28K, $renP_{CD3}$, $renP_{CD6}$, cytokeratin 19, TROMA-I, TROMA-III, fibrillin-1, laminin γ1, transglutaminase II, ezrin, and agrin.

Generation of rows of tubules by piling

The second series of culture experiments were performed to increase the amount of tubules by piling embryonic renal tissue (Fig. 4). A schematic illustration depicts that each layer of embryonic renal tissue was separated by a layer of polyester fleece to produce the interphase of an artificial interstitium (Fig. 4A). Low magnification of toluidine blue–stained cryosections revealed that, at a distance between 500 and 600 µm, two parallel layers of tissue developed after a 13-day culture period (Fig. 4B). Histochemistry further demonstrated that both layers of generated tissue could be labeled by SBA (Fig. 4C) and TROMA-I (Fig. 4D). Higher magnification of specimens stained for TROMA-I (Fig. 4E), SBA (Fig. 4F), and DBA (Fig. 4G) exhibited numerous developed tubules in longitudinal and cross-sectional views. The label for epithelium-specific E-cadherin further demonstrated that lining cells were developed within the tubules (Fig. 4H).

Microscopic observation of generated tissue after histochemical labeling under low microscopic magnification led to bleaching of the fluorescence marker within the shortest time. A compromise was to analyze both rows of tubules separately (Fig. 5). Histochemical labeling of individual rows of stacked tubules showed an intensive signal for SBA (Fig. 5A–A″), TROMA-I (Fig. 5B–B″), DBA (Fig. 5C, C′), COX 2 (Fig. 5D, D′), and E-cadherin (Fig. 5E, E′).

DISCUSSION

To date, it is unknown which cellular biological mechanisms trigger the development of renal stem/progenitor cells from single cells toward structured tubules.[24,25] This process comprises epithelialization, tube formation, and its extension in a spatial environment. Thus, the present

FIG. 2. Schematic (**A**) and whole-mount views (**B**) of generated tubules derived from a single layer of embryonic renal tissue. In a microscopic opening of 620×930 µm, between 55 and 77 tubules were found after SBA label. Generated tubules exhibited a basal lamina and a lumen (**C**). Both longitudinal (**D**) and vertical (**E**) views showed a basal lamina and a lumen. Asterisk: basal lamina; arrow: lumen. Color images available online at www.liebertpub.com/ten.

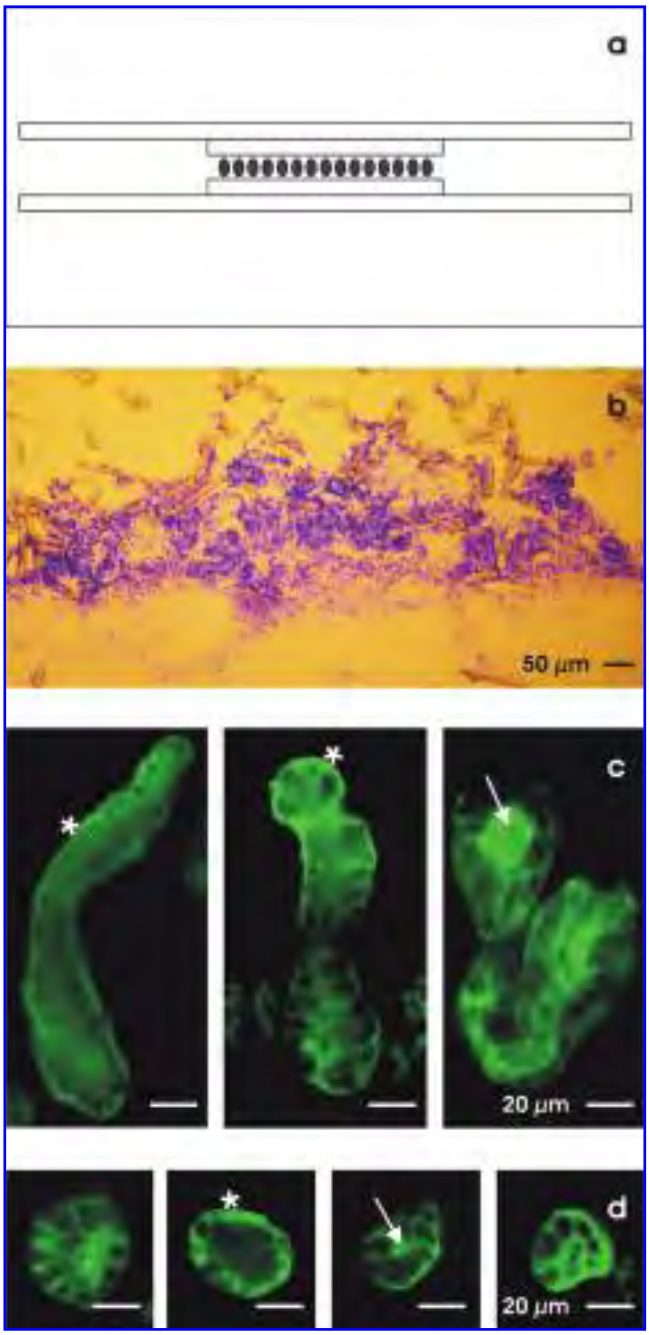


FIG. 3. Cryosections of generated tubules derived from a single layer of embryonic renal tissue (**A**). Surface view of toluidine blue–stained renal tissue cultured for 13 days at the interphase of an artificial interstitium (**B**). Numerous tubules embedded in loose connective tissue are recognized in cross sections and longitudinal view. Longitudinal view (**C**) and cross sections (**D**) of tubules labeled with SBA exhibited a basal lamina and a lumen. Asterisk: basal lamina; arrow: lumen. Color images available online at www.liebertpub.com/ten.

experiments should elucidate whether it is possible to extend the spatial environment for the development of renal stem/progenitor cells. Since the creation of an artificial interstitium revealed optimal tubule formation, renal stem/progenitor cells–containing tissue was isolated (Fig. 1A) and placed between layers of polyester fleece (Fig. 1B, C).[18–20] This specific experimental setup made it possible to pile and pave renal stem/progenitor cells like bricks and extend the spatial formation of tubules. An advantage of this system was that it could be performed at the interphase of polyester fleeces. With this technique, it was unnecessary to coat the fleece with extracellular matrix proteins. Perfusion culture with fresh and chemically defined medium further guaranteed the constant nutrition and exchange of respiratory gases during a 13-day culture period. Interestingly, the development of tubules was dependent on aldosterone application. The steroid hormone revealed tubulogenic activity, acted in a concentration-dependent manner and could be blocked by antagonists such as spironolactone and canrenoate.[23,26,27]

Culturing single rows of renal stem/progenitor cells resulted in between 55 and 77 SBA-labeled tubules, as detected in a microscopic opening with a measurement of $620 \times 930\,\mu m$ (Fig. 2B). SBA-labeled tubules were not detected at initiation of culture. Generated tubules grew not guided but accidentally distributed and appeared densely packed. SBA labeling further demonstrated that the generated tubules gained features of specific differentiation, which were not present at the start of culture. The analyzed tubules exhibited a basal lamina, lining polarized cells and a clearly visible lumen (Fig. 2C–E). Aside from SBA, numerous other cell biological markers were detected on generated tubules (Table 1). We have not shown data about functionality yet. However, the primary upregulation of tight junctions between the luminal and lateral plasma membranes or the upregulation of Na/K-ATPase were clear hints for functional development. Hence, experiments related to transport, metabolic activity, and ammoniagenesis of generated tubules will be in the focus of future work.

Markers detected on generated tubules could be ascribed to two different groups. DBA, GSL, UEA I, VVL, and WGA or functional proteins such as desmoplakin I/II, Na/K-ATPase, COX 2, calbindin-D-28K, renal P_{CD3},[28] renal P_{CD6},[28] fibrillin-1, and ezrin demonstrated that primary expression of these molecules occurred during culture. Freshly isolated renal stem/progenitor cells were completely negative for these markers, while tubules generated in culture were positive. In contrast, markers like occludin, E-cadherin, TROMA-I, TROMA-III (cytokeratin 19), laminin γ1, transglutaminase II, and agrin demonstrated positive labeling in freshly isolated collecting duct ampullae as in generated tubules. These results indicated that typical features were already present initially at isolation and remained present until the end of culture. In addition, the set of tested markers demonstrated that generated tubules derived from collecting duct (CD) ampulla cells and gained features of the matured renal CD.

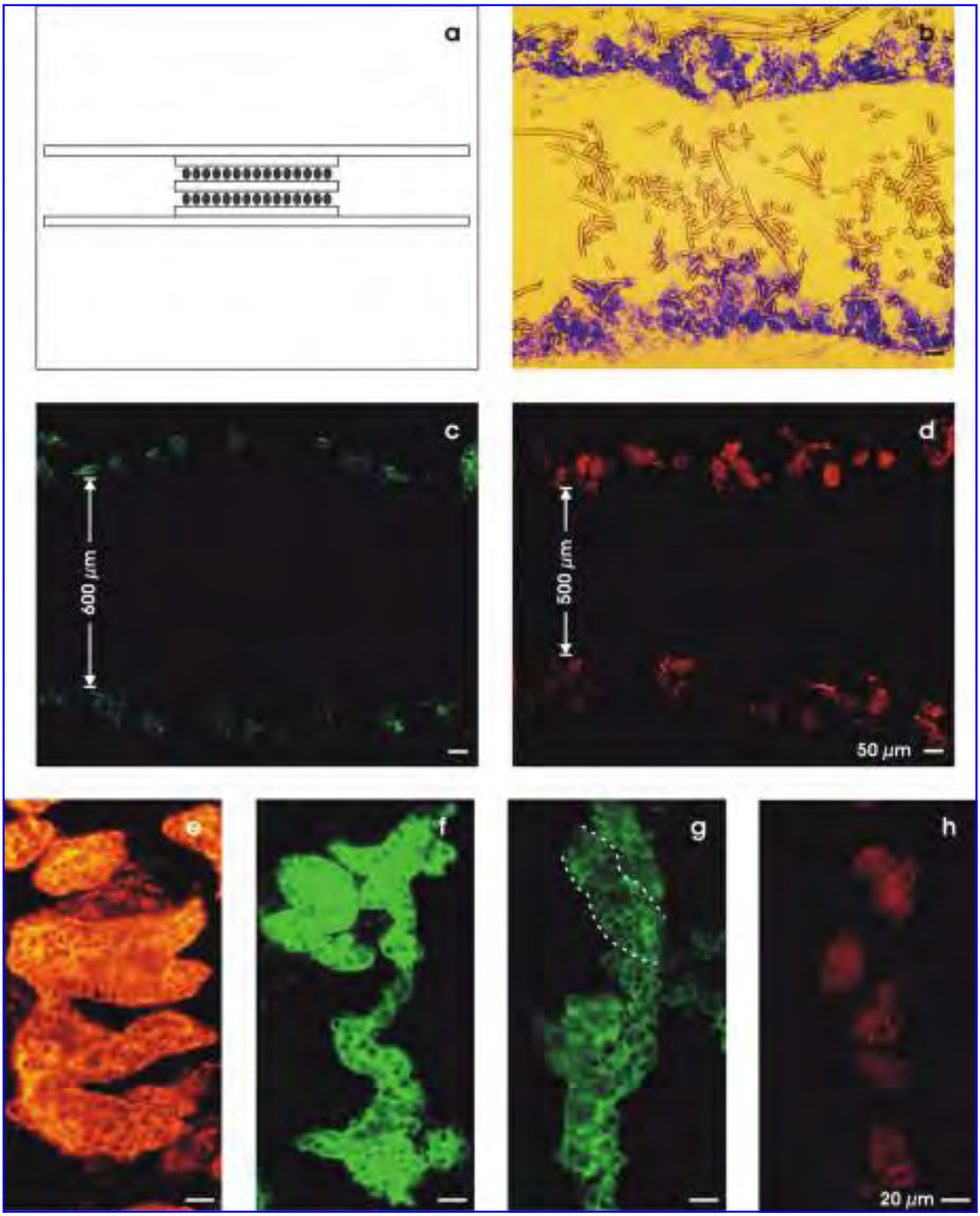


FIG. 4. Piling up renal tubules. Cryosections of generated tubules derived from two layers of embryonic renal tissue (**A**). Toluidine blue staining of tissue cultured for 13 days at the interphase of an artificial interstitium (**B**). Two layers of generated structures are visible. SBA labeling demonstrates features of generated tubules (**C**). The distance between the rows is 600 µm. TROMA-I label shows the presence of two rows of numerous tubules (**D**). The distance between the rows is 500 µm. Label for TROMA-I (**E**), SBA (**F**), and DBA (**G**) depicts features of generated tubules. Dotted line represents frontier between tubules lining in parallel. Labeling for E-cadherin demonstrates presence of lining epithelial cells (**H**). Color images available online at www.liebertpub.com/ten.

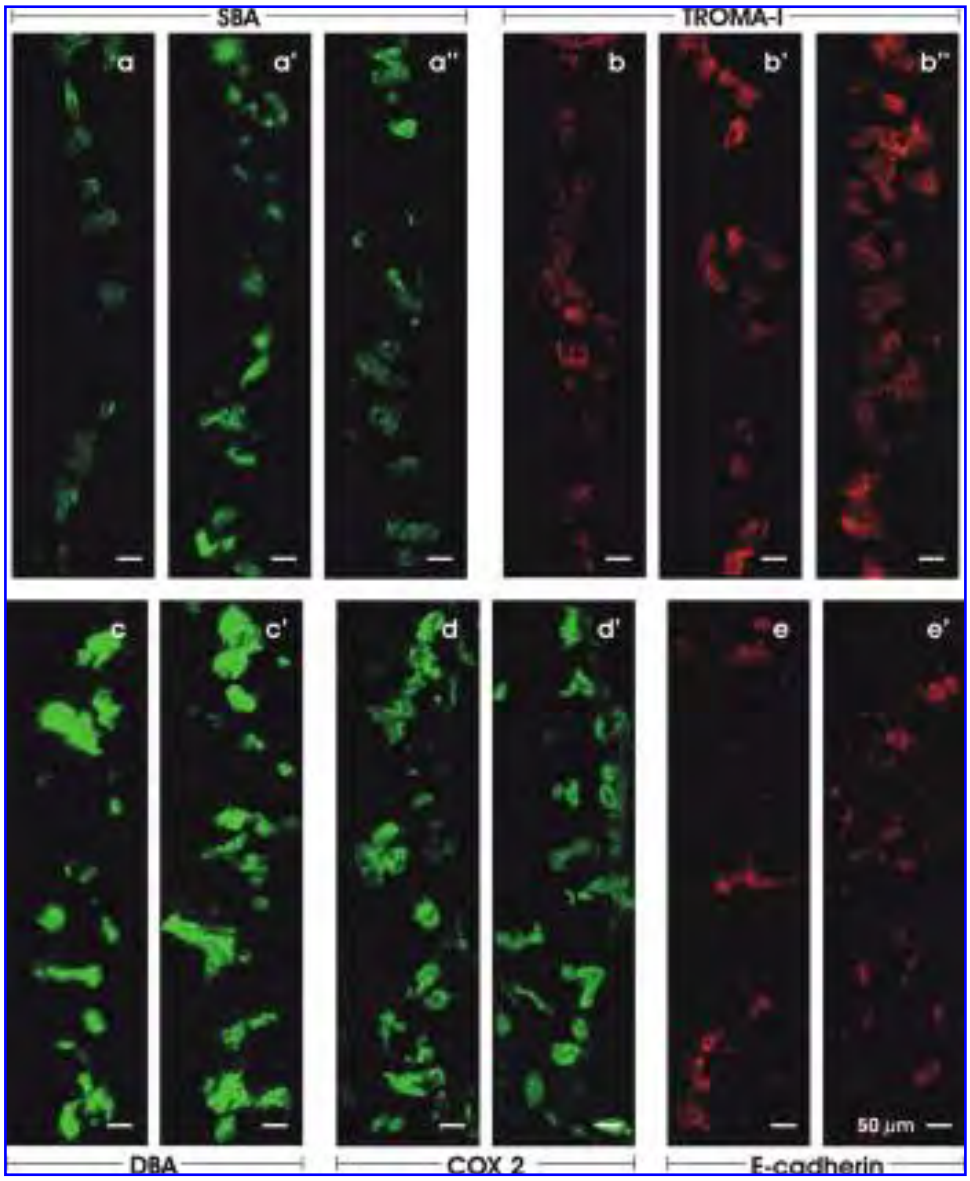


FIG. 5. Piling renal tubules. Histochemistry of generated tubules derived from two layers of embryonic renal tissue. SBA labeling demonstrates features of generated tubules (**A–A″**). TROMA-I (**B–B″**), DBA (**C, C′**), COX 2 (**D, D′**), and E-cadherin (**E, E′**) labeling demonstrates the presence of numerous developed tubules. Color images available online at www.liebertpub.com/ten.

Culturing renal stem/progenitor cells at the interphase of an artificial interstitium made piling and paving, for the first time, possible. Using this innovative approach, the spatial environment for developing renal tubules could be extended experimentally step by step. For the present experiments, renal stem/progenitor cells were initially maintained as a basic sandwich setup, a single tissue layer between two layers of polyester fleece (Fig. 6A). To extend the plane for growth, basic sandwich setups were paved by placing them side by side (Fig. 6B, microscopic illustration is not shown). To increase the spatial environment, basic sandwich setups were stacked so that parallel rows of tubules could develop (Fig. 6C). A combination of piling and paving the initial amount of renal stem/progenitor cells revealed that spatial tubule formation could be further increased (Fig. 6D). Using three or more basic sandwich setups for piling (Fig. 6E) in combination with paving (Fig. 6F), a further extended spatial environment for the growth of tubules could be achieved.

The present experiments demonstrated that parallel rows of generated tubules could be obtained when renal stem/progenitor cells were piled (Figs. 4 and 5). Labeling

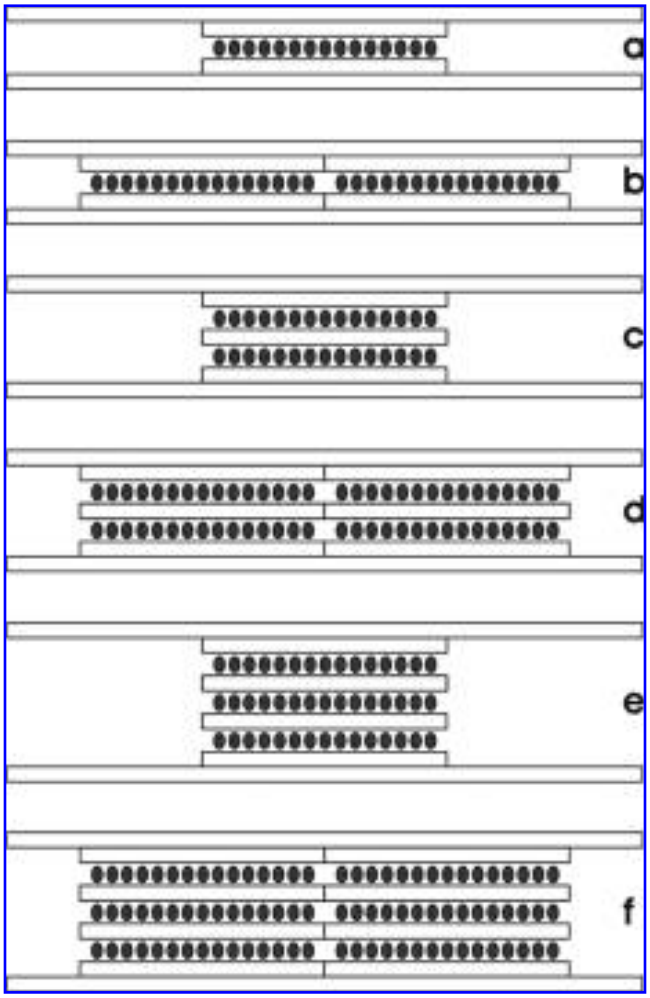


FIG. 6. Systematic steps for the creation of renal superstructures. A single layer of embryonic renal tissue was cultured at the interphase of an artificial interstitium (**A**). By paving, the area of growth can be increased (**B**). By piling, the height of generated tissue can be increased (**C**). Combination of piling and paving increased the volume of generated tissue (**D**). Further piling (**E**) and paving (**F**) consequently leads to built up of renal superstructures.

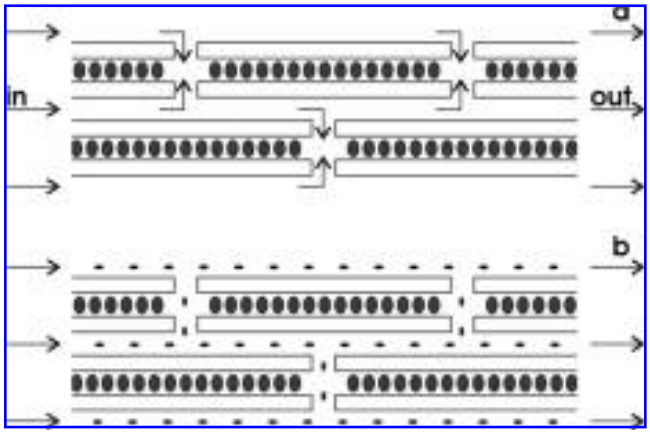


FIG. 7. Piling embryonic renal tissue provides advantages for nutrition. The supply between the tissue layers is optimal since nutrition and respiratory gases are continuously exchanged through a wide space located between the polyester fibers (**A**). Piling may be used for coculture with endothelial cells supporting vascularization after implantation (**B**).

generated tubules by histochemical markers further revealed that the same amount and same degree of differentiation were found in piled specimens (Figs. 4 and 5), as observed in experiments with single rows of renal stem/progenitor cells (Figs. 2 and 3). Thus, these experiments made it feasible to pave and pile basic sandwich setups containing renal stem/progenitor cells like bricks to extend the spatial environment for the growth of tubules.

The arrangement of the basic sandwich setup was most advantageous for culture medium supply. The developing tissue was covered by layers of polyester fleece. Fresh medium was continuously supplied through the space between the fibers of the polyester fleece separating the tissue layers (Fig. 7A). With the permanent exchange of fluid using perfusion culture, the unstirred layers were minimized. Thus, piling and paving allowed the medium to pass freely between the tissue layers and the polyester fleeces.

In future, the use of an artificial interstitium in combination with piling basic sandwich setups could be advantageous for microvascularization (Fig. 7B). Endothelial cells

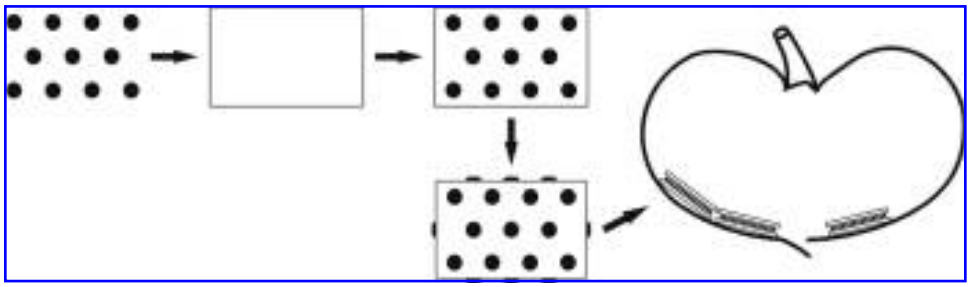


FIG. 8. A favorable site for implantation of constructs appears to be beyond the renal capsule. This is the region where renal development is terminated after the organ reaches its final size.

could be added to the culture medium and infused along the fluid path within the polyester fleeces. The presence of widely distributed endothelial cells would consequently support rapid vascularization of the constructs.

The advantages of piling and paving basic sandwich setups containing renal stem/progenitor cells are numerous (Fig. 7). One could imagine that superstructures derived by a combination of piling and paving numerous basic sandwich setups can be achieved to generate organoid structures under *in vitro* conditions. Therefore, it is imaginable that the procedure for piling and paving is not performed as in our experiments by forceps but could be processed in a computerized robotic process. Such superstructures could be applied to investigate the development of organoid structures under controlled *in vitro* conditions and generate tissue constructs for minimally invasive implantation into a diseased kidney.

An important challenge for the future will be to find the optimal site for implantation of developing renal stem/progenitor cells into diseased human kidneys.[29] We would favor an implantation site beyond the renal capsule (Fig. 8). At this site, renal development was terminated when the organ reached its final size after growth. In adults this site would be easily accessible for minimally invasive procedures. However, experiments have to be performed on animals to elaborate parameters for minimal cell mass required to stimulate the regeneration process. In addition, more information about the molecular processes involved in the development of renal parenchyma in a physiological and pathophysiological context is necessary. Only detailed knowledge in this special experimental field will make it possible to find successful therapeutic protocols for the regeneration of tubules.

CONCLUSIONS

In the present set of experiments, renal stem/progenitor cells were cultured at the interphase of an artificial interstitium made of polyester fleeces in perfusion culture. This special experimental configuration made it possible to pile and pave renal stem/progenitor cells and to extend the spatial environment for the formation of tubules. It could be demonstrated that piling of renal stem/progenitor cells resulted in parallel rows of developed tubules. Histochemical labeling indicated a high degree of differentiation. Thus, piling and paving renal stem/progenitor cells appears to be a successful step in tissue engineering for the systematic spatial development of tubules found within the renal parenchyma.

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