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Cell and drug delivery therapeutics for controlled renal parenchyma regeneration ☆

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ABSTRACT

In regenerative medicine much attention is given to stem/progenitor cells for a future therapy of acute and chronic renal failure. However, up to date sound cell biological knowledge about nephron renewal in kidney is lacking. For that reason molecular mechanisms are under intense investigation leading from stem/progenitor cells to regenerated tubules. In this coherence new biomaterials and drug delivery systems have to be elaborated showing an intense stimulation on the renewal of parenchyma.

To analyze tubule regeneration a powerful culture system is of fundamental importance. An advanced technique stimulates renal stem/progenitor cells to develop numerous tubules between layers of a polyester fleece. Use of chemically defined Iscove's Modified Dulbecco's Medium (IMDM) containing aldosterone (1×10^{-7} M) results in spatial development of renal tubules within 13 days of perfusion culture.

Immunohistochemistry exhibits that numerous features of a polarized epithelium are expressed in generated tubules. Transmission electron microscopy (TEM) illuminates that generated tubules contain a polarized epithelium with a tight junctional complex and an intact basal lamina at the basal aspect.

Development of tubules depends on applied aldosterone concentration and cannot be mimicked by precursors of its synthesis pathway or by other steroid hormones. Antagonists such as spironolactone or canrenoate prevent the development of tubules. This result illuminates that the tubulogenic development is mediated via the mineralocorticoid receptor (MR). Application of geldanamycin, radicicol, quercetin or KNK 437 in combination with aldosterone blocks development of tubules by disturbing the contact between MR and heat shock proteins 90 and 70.

In conclusion, for the first time generation of renal tubules can be simulated under controlled in-vitro conditions. Using this model the effect of numerous innovative biomaterials and drug delivery system can be critically analyzed.

Contents

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1. Introduction

The incidence of chronic kidney disease is growing since years, largely as a result of increased prevalence of diabetes and obesity. It is estimated to affect 11% of the adult population [1]. In contrast to liver the kidney has been classically regarded as an organ with minimal cell turn over and lacking capacity for regeneration [2,3]. The dogma tells that the kidney reaches during organ growth a maximal number of nephrons, which are diminished over time [4,5]. This would mean that a replacement of parenchyma lost in its original form does not occur in the mammalian kidney.

Although true regeneration is not thought to occur, the kidney does maintain a capacity to undergo repair after damage [6–8]. For example, even after prolonged unilateral ureteric obstruction, involved inflammation and necrosis the renal cortex is able for substantial remodelling [9]. Up to date it is unknown, if the regeneration is made by parenchymal cells undergoing self renewal, by interstitial cells performing mesenchymal-epithelial transitions or by an activation of resident or invading stem/progenitor cells. There are convincing arguments for both that endogenous but also exogenous stem/progenitor cells might be the source for regenerating parenchyma [8].

The site of repair in a diseased kidney is harmful. Regenerating cells are exposed to an environment, where tubule cells undergo necrosis and apoptosis leading to a detachment including denudation of the basal lamina [10]. Here a process of degradation has to be terminated and turned into a process of regeneration. The presupposition for a successful therapeutically induced regeneration is therefore the recreation of an optimal microenvironment suppressing inflammation but promoting cell proliferation and differentiation including the subsequent spatial development of functional tubules [11–13].

In this coherence the question arises, which molecular processes hamper a diseased kidney to regenerate parenchyma and to which extend such a process might be stimulated by newly developed pharmaceuticals [14]. Finding the right answers to these questions the actual research is focusing on the one hand to innovative biomaterials supporting the spatial development of parenchyma and on the other hand to hormones, growth factors and morphogens promoting the process of regeneration [15,16].

Research related to regeneration and compensation of renal parenchyma is an up to date still barely developed area. In contrast to the adult organ much more information was generated in multiple papers dealing with the kidney anlage and the reciprocal interactions between the ureter bud and the nephrogenic mesenchyme [17–19]. Numerous data are available concerning the primary steps of organogenesis and the formation of S-shaped bodies as first visible signs of nephrogenesis [20,21]. However, surprisingly little knowledge is on hand about subsequent cell biological mechanisms involved in spatial tubule formation. The development of renal parenchyma is more complex than frequently assumed. For example, except for phosphoinoside-3-kinase [13], mTOR signalling [22] and Crumbs complex [23] the molecular mechanisms leading to nephron segmentation and maturation are unknown [24]. Also the proceeding formation into a spatially structured tubule is up to date not understood [25]. These pleiotropic steps of development comprise the sprouting of cells, the formation of a lumen and the elongation of the individual tubule segments. During the proceeding developmental steps a highly specialized epithelium is integrated into a tubule exhibiting exact geometrical dimensions such as length, inner and outer diameter [26,27]. For renal tubules it is not known, if the formation of a lumen occurs by wrapping, budding, cavitation or hollowing [28]. Finally, during the phase of organ expansion spatial features such as straight course, length, convolution or eventual branching of a tubule are determined so that finally the complex microarchitecture of the kidney is arising.

2. Finding a powerful model for in vitro regeneration

Due to the modest knowledge of spatial tubule development intense basic research has to be performed. On the one hand learning about the program of cellular differentiation is essential and on the other hand molecular insights have to be gained in the versatile processes promoting the spatial construction of functional tubules [21,29–31]. In addition, the three-dimensional organization of the kidney has to be considered including the multiple growth factors triggering the single steps of development. Obtaining exact information about the molecular processes of tubule formation new pharmaceuticals are needed that may accelerate the progress of regeneration [32].

The complex microarchitecture of the kidney hinders to perform experiments in situ. For that reason sophisticated culture systems are needed to elaborate the necessary information about spatial tubule development. A simple culture experiment elucidates that development of renal tubules cannot be expected, when a panel of essential environmental parameters is missing in the dish (Fig. 1a). For example, renal tubule segments were isolated and placed at the bottom of a culture dish [33–36]. Most interestingly, addition of a culture medium containing fetal bovine serum does not result in the elongation of the isolated tubule. In contrast, cells start to migrate, leave the interior of the tubule to spread on the bottom of the culture dish and on the outer side of the basal lamina. In each case most of the cells do not remain on the original position. In conclusion, an isolated tubule is not able to extend its length and the cells do not remain on the site of origin. This uncontrolled migration of cells is induced by spreading factors contained in serum. It leads to a loss of polarization and to a misleading adhesion of cells on the polystyrene buttom of the culture dish lining in parallel with a process of dedifferentiation.

To offer an improved substitute for the basal lamina and to support polarization renal epithelial cells are frequently cultured on a filter (Fig. 1b). In contact with a selected filter the cells show an unexpectedly sensitive reaction [37–40]. The degree of differentiation depends on the selected filter material, its surface structure, on pore size and on coating by extracellular matrix proteins. All of these factors together determine, if a confluent growth in combination with an optimal polarization of cells will be obtained.

The culture of epithelial cells on the bottom of a dish or on a filter results in a two-dimensional spreading, which is not typical for the arrangement within a tubule. In consequence, to obtain a three-dimensional orientation isolated cells collected from the urine [41,42] or MDCK cells [43–46] were coated at the start of culture by extracellular matrix proteins such as collagen or matrigel (Fig. 1c) [47–50]. After initiation of culture cells migrate to form cords. After a few days spatial formation of tubules is observed. By the first view the coating by extracellular matrix proteins appears as an optimal technical solution. However, the period of culture time is limited, since the relatively thick layer of three-dimensional coating hinders an optimal exchange of nutrition and respiratory gas by diffusion (Fig. 2a). After a

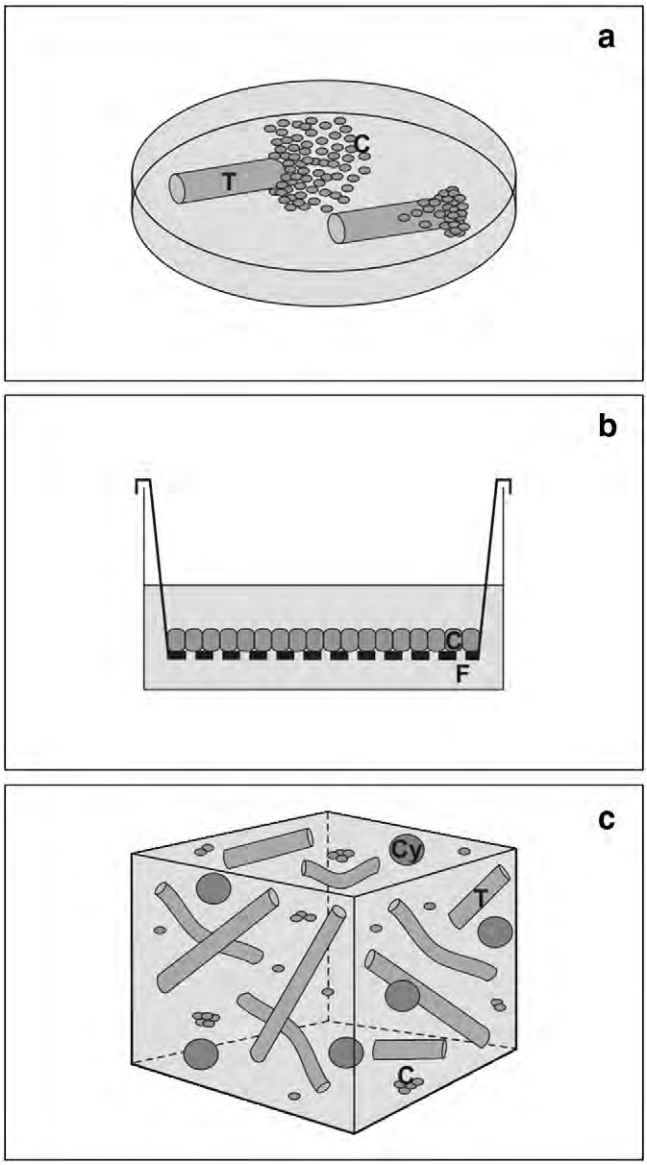

Fig. 1. Renal cells in culture. (a) When isolated tubules (T) are kept in serum-containing medium cells (C) emigrate and sprout at the bottom of a culture dish or on the outer surface of the tubule. (b) Culture within a filter (F) insert. Polarization depends on selected filter material, pore size and surface coating. (c) Coating of cells with extracellular matrix proteins stimulates the formation of cell rows, cysts (Cy) and tubules (T).

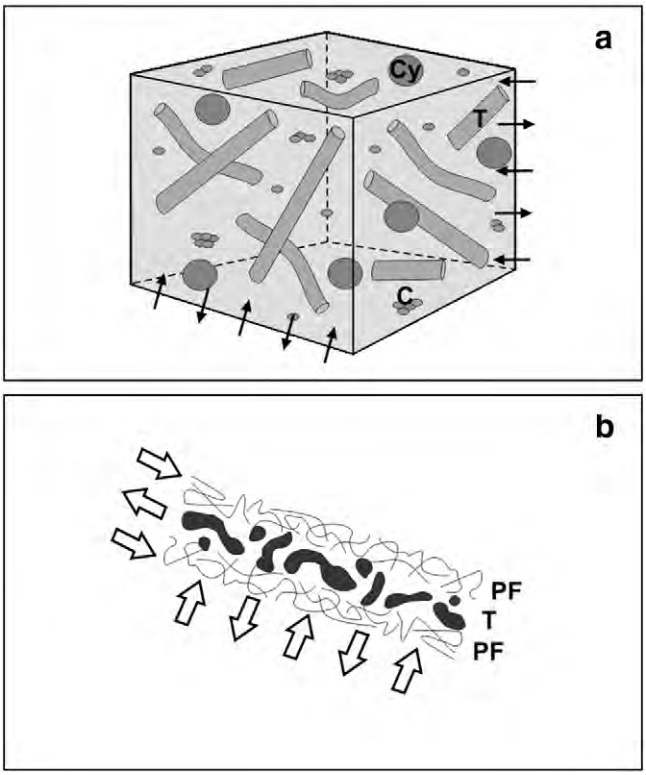

Fig. 2. Coating by extracellular matrix proteins versus creation of an artificial interstitium. (a) Coating the cells (C) by extracellular matrix proteins during long term culture is problematic, since it hinders optimal exchange of nutrition and respiratory gas (small arrows) leading to formation of unstirred layers of medium and causing in turn deleterious accumulation of metabolites. (b) An artificial interstitium made of a polyester fleece (PF) supports the exchange of nutrition including respiratory gas and promotes the spatial development of tubules (T) (dark arrows).

few days the coating by extracellular matrix proteins leads to the formation of unstirred layers of medium. This effect again causes a deleterious accumulation of metabolites, which in turn harms the cells by a suboptimal culture environment during prolonged periods of time. Problematic is further the use of individual coating solutions, when they are prepared out of tissue derived from animals or tumor cells such as Engelbreth-Holm-Swarm (EHS) mouse sarcoma [51]. Undefined molecules, viral and bacterial pathogens may be contained. For these reasons coating by extracellular matrix proteins of uncertain origin is not performed in our laboratory.

3. Creating an artificial interstitium

For the spatial development of tubules the in vitro environment has to meet the very special needs of renal stem/progenitor cells. In the kidney this specific environment is presented by the interstitium. It consists of both extracellular matrix fibers and nutritional fluid including respiratory gas. The aim of the presented investigations was therefore to create in the culture experiments such an interstitium as it is found within the kidney [52,53]. Stem/progenitor cells should be exposed to a suitable biomaterial promoting spatial development, exhibiting a certain mechanical stiffness and providing the necessary nutrition and respiratory gas. The technical solution for the creation of an artificial interstitium is to culture stem/progenitor cells between layers of a polyester fleece. The fibers of the fleece simulate the extracellular matrix, while the space between them is accessible for the transport of culture medium and respiratory gas (Fig. 2b). Numerous performed experiments show that the applied polyester fleece supports the continuous exchange of medium over long periods of weeks during perfusion culture. Most important, the interface between the fleece layers exhibits up to date unknown features promoting the spatial development of tubules.

4. Using a suitable cell source

To investigate basic mechanisms of renal tubule regeneration under in vitro conditions a suitable source of renal stem/progenitor cells is needed. Due to the limited size of embryonic mouse or rat specimens, neonatal rabbit kidney is selected as a favorite model (Fig. 3). Even after birth the embryonic cortex of the organ contains numerous stem cell niches within their original extracellular environment [54]. The niche of stem/progenitor cells can be shown in the outer cortex in close neighborhood to the organ capsule. Two different kinds of stem/progenitor cells are found at this site. Epithelial stem/progenitor cells are detected within the tip of the collecting duct ampulla. The basal aspect of each ampulla is surrounded by numerous mesenchymal nephrogenic stem/progenitor cells. Reciprocal interaction between both results in a Comma-shaped

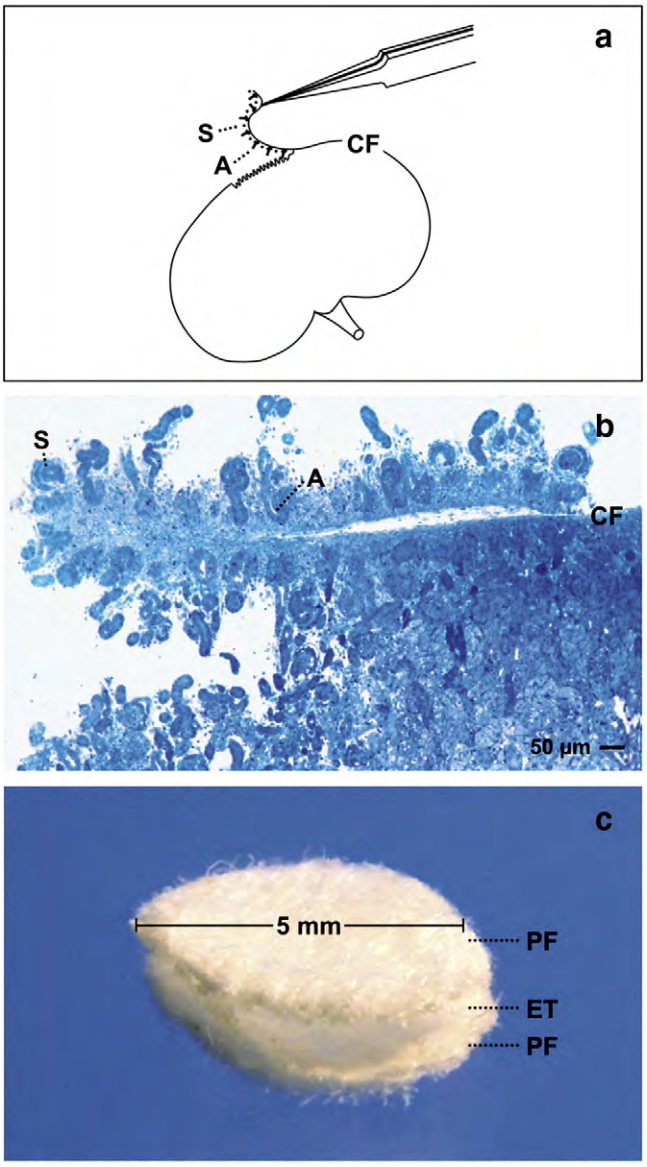

Fig. 3. Isolation of renal stem/progenitor cells from neonatal rabbit kidney. (a) Beyond capsula fibrosa (CF) epithelial stem/progenitor cells are found within the collecting duct ampullae (A), while nephrogenic mesenchymal stem/progenitor cells are located in the close vicinity. S-shaped bodies (S) are recognized as first signs of nephron development. (b) For culture the embryonic tissue layer is stripped off by fine forceps consisting of capsula fibrosa (CF), ampulllae (A) and S-shaped bodies (S). (c) Fotografic illustration shows that the embryonic tissue (ET) is mounted between two layers of a polyester fleece (PF) to create an artificial interstitium.

and then in a S-shaped body as first morphological signs of starting nephron development [20,55]. Although communicating epithelial and mesenchymal stem/progenitor cell populations do not stand in close contact to each other but are separated by an astonishingly wide interstitial space [56].

An advantage for culture is that the embryonic tissue layer beyond the organ capsule of neonatal rabbit kidney can be easily isolated (Fig. 3a,b) [54]. Stripping off the capsule with fine forceps, a thin layer of tissue adheres to the explant. It contains both epithelial stem/progenitor cells and nephrogenic mesenchymal stem/progenitor cells beside some comma-shaped and S-shaped bodies. Applying this simple isolation method an embryonic tissue layer of up to 1 cm^2 in square can be harvested. Up to date no other species is known for the isolation of tissue containing renal stem/progenitor cells in such an amount necessarily needed for subsequent culture experiments and cell biological analysis.

5. Mounting a tissue carrier

After isolation the embryonic tissue is placed between two layers of polyester fleece (I7, Walraf, Grevenbroich, Germany), which are punched out to discs measuring 5 mm in diameter (Figs. 3c and 4a). This specific arrangement results in a basic sandwich set-up configuration with the freshly isolated embryonic tissue in the middle and layers of polyester fleece covering the upper and lower sides [57].

To prevent damage during culture the basic sandwich set-up containing renal stem/progenitor cells must be held in an exact position inside a perfusion culture container (Fig. 4a). For mounting a base ring of a Minusheet® tissue carrier (Minucells and Minutissue, Bad Abbach, Germany) with 13 mm inner diameter is used (Fig. 4b). First a polyester fleece measuring 13 mm in diameter is placed in the tissue carrier. Then the basic sandwich set-up measuring 5 mm in diameter is mounted. Finally, another polyester fleece 13 mm in diameter is transferred to the top of the sandwich as a cover. For perfusion culture the tissue carrier is used in a container with horizontal flow characteristics (Minucells and Minutissue, Bad Abbach, Germany) (Fig. 4c). By closing the lid of the perfusion container the basic sandwich set-up is held in an exact position.

6. Providing always fresh culture medium

To maintain a constant temperature of 37°C, the perfusion culture container is placed on a thermoplate (Medax-Nagel, Kiel, Germany) and covered with a transparent lid. For a culture period of 13 days always fresh medium is transported by a rate of 1.25 ml/h with an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany) under atmospheric air (Fig. 4d). Applying this method medium is saturated to 160 mmHg oxygen during transportation [58]. The high content of oxygen in the medium is reached by a long thin-walled silicone tube, which is highly gas-permeable guaranteeing optimal diffusion between culture medium and surrounding atmosphere. In this way it is possible to adjust the gas partial pressures within the medium under absolutely sterile conditions. By running under atmospheric air or by maintaining a defined CO_2-concentration this method can be employed to control medium pH, when a biological buffer such as HEPES or Buffer All is added to the medium.

For the generation of renal tubules chemically defined IMDM (Iscove´s Modified Dulbecco´s Medium including Phenolred, GIBCO/Invitrogen, Karlsruhe, Germany) is used in described experimental series [59]. In order to maintain a constant pH of 7.4 under atmospheric air containing 0.3% CO_2 HEPES (50 mmol/l, GIBCO/Invitrogen) is added to the medium. Infections are prevented by an antibiotic-antimycotic cocktail (1%, GIBCO). To induce tubulogenic development aldosterone (1×10^{-7} M, Fluka, Taufkirchen, Germany) is administered in the storage bottle [60]. Application of serum or other undefined ingredients is not performed.

7. Collecting generated tubules

After a perfusion culture period of 13 days the tubules are matured so that a cell biological analysis can be performed. By tearing off the layers of the fleece the artificial polyester interstitium is opened. The area for tubule formation is 5 mm in diameter and up to 250 µm in height. For whole mount labeling the specimens are fixed in ethanol and stained by fluorescent Soybean Agglutinin (SBA) (Fig. 5a). This screening procedure can be easily performed and saves time as compared to immunohistochemistry on cryosections. It results in a perfect surface view of generated tubules.

Freshly isolated stem/progenitor cells do not exhibit SBA-label. Acquisition of SBA-binding on cells after a 13 days culture period in IMDM containing aldosterone (1×10^{-7} M) illustrates an important step in development. To obtain exact information about the number of generated tubules a WCIF ImageJ program (Bethesda, Maryland, USA)

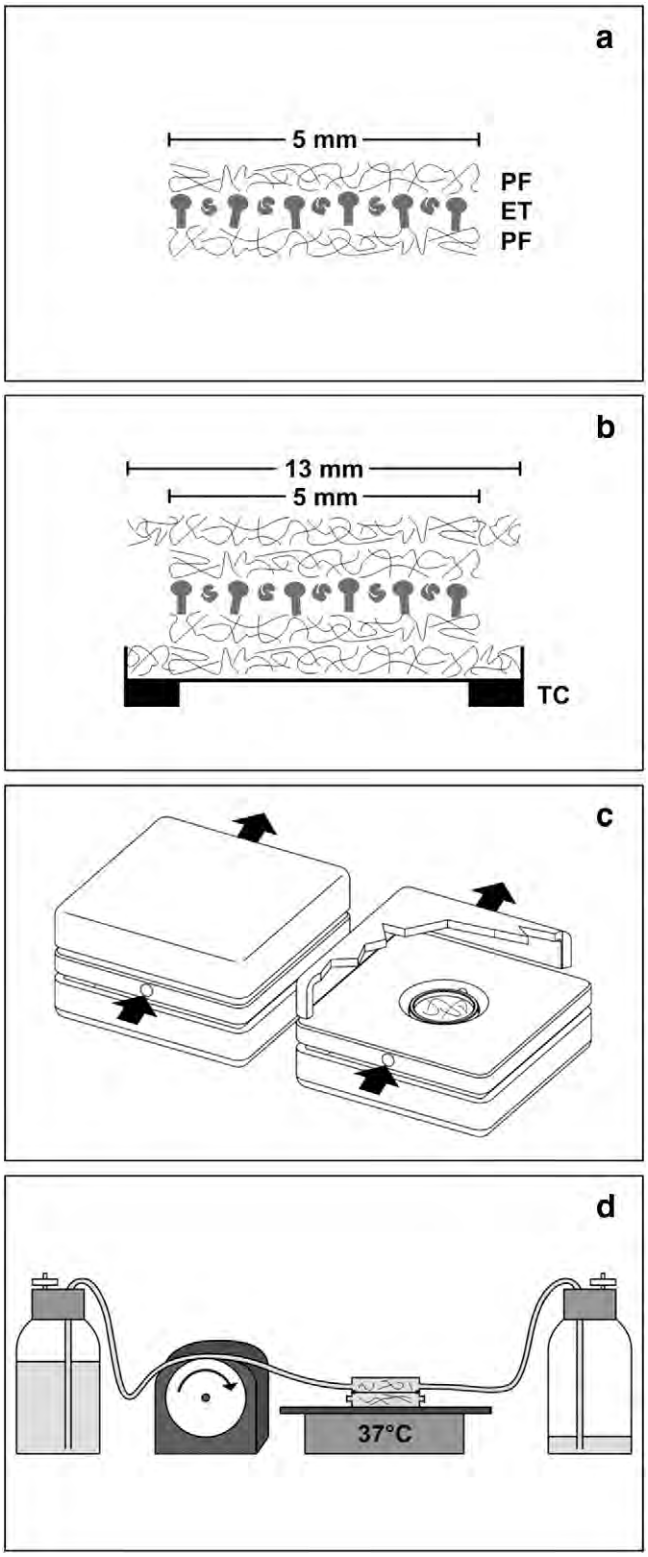

Fig. 4. Long term culture at the interface of an artificial interstitium. (a) Schematic illustration depicts that an artificial interstitium is made by placing isolated embryonic tissue (ET) between two layers of polyester fleece (PF). (b) The basic sandwich set-up containing renal stem/progenitor cells is mounted in a tissue carrier (TC). (c) For culture the tissue carrier is placed in a perfusion culture container containing a medium inlet and outlet (arrows). (d) Perfusion culture is performed with a peristaltic pump transporting always fresh medium (1.25 ml/h) from the storage bottle (left) to the culture container. Used medium is not recycled but is collected in a waste bottle (right). Temperature is maintained by a thermoplate.

is used for counting SBA-labeled tubules. Applying this technique an individual example depicts that 100 tubules are detected within a microscopic opening of 840 × 500 μm (Fig. 5b). Fluorescence microscopy of SBA-labeled specimens further demonstrates that tubules are growing in a spatial arrangement. Part of it illustrates a straightforward growth, while others reveal a dichotomous branching or curling. When the tubules are not leaving the optical plain, it is possible to follow their longitudinal growth over a distance between 300 and 400 μm. Whole mount label further shows that generated tubules exhibit polarized cells, a visible lumen and a basal lamina. In so far the used I7 polyester fleece is providing a perfect interaction with renal stem/progenitor cells during perfusion culture resulting in spatial development of numerous tubules.

8. Illustrating development

To obtain more detailed information about features of up-regulated proteins cryosections of generated tubules were analyzed after a 13 days culture period by immunohistochemistry. Positive reaction for cingulin, Na/K ATPase α5, laminin γ1, and collagen III exhibits an intense label on all tubules. Label for cingulin demonstrates the development of a tight junctional belt recognized as faint reaction in the luminal portion of generated tubules (Fig. 5c). Bright labeling for Na/K ATPase α5 is detected at the basolateral plasma membranes (Fig. 5d). Strong reaction for laminin γ1 indicates the presence of this typical protein in the basal lamina (Fig. 5e). Finally, immunohistochemical label for collagen type III is contained in both the basal lamina of generated tubules and in the surrounding interstitial space (Fig. 5f). This result illuminates synthesis of extracellular matrix in form of collagen III as import interstitial protein between generated tubules.

9. Focusing to the interstitial side of tubules

The use of a polyester fleece as artificial interstitium replaces coating of cells by extracellular matrix proteins at the start of culture. Applying the artificial interstitium it became for the first time possible to analyze the basal aspect of generated tubules by scanning electron microscopy (SEM) [61]. Using an artificial polyester interstitium the view to the basal aspect of generated tubules is not stacked by extracellular matrix proteins derived from a coating process. Thus, SEM illustrates development of numerous tubules (Fig. 6a). The surface view depicts that the generated tubules have only a loose contact with the fibers of the polyester fleece. Many of the tubules develop in a parallel fashion, while others exhibit curling or dichotomous branching. All of the tubules are covered by a continuously developed basal lamina. On the surface of the basal lamina interstitial cells and bundles consisting of newly synthesized extracellular matrix proteins are recognized.

For control, SEM of the artificial interstitium at sites without tubule development shows numerous polyester fibers running in a longitudinal, transversal and oblique course. The fibers appear to be of homogeneous composition and exhibit a smooth surface without recognizable protrusions or roughness. The average diameter of a polyester fiber is 10 μm. Chemical cross-linking between the fibers of the fleece cannot be observed.

10. Regarding ultrastructural features

To obtain insights in the ultrastructure of generated tubules, transmission electron microscopy (TEM) was performed [62]. A surface view reveals that tubules are developing in the neighborhood of polyester fibers, however, avoiding close contact to them. In the surrounding of a tubule synthesized extracellular matrix fibers, single interstitial cells and cellular debree are recognized.

Generated tubules contain an isoprismatic epithelium surrounding a lumen (Fig. 6b). In the center of a cell a large nucleus is located.

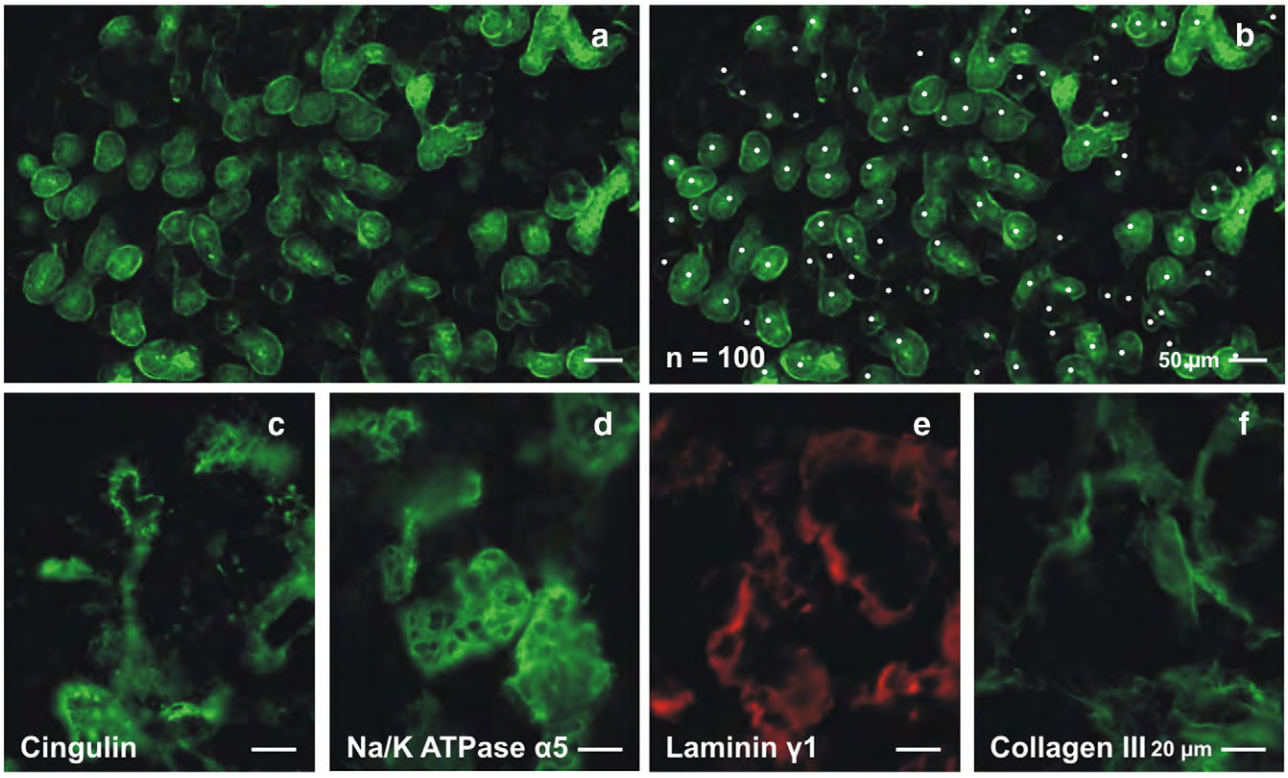

Fig. 5. Generated renal tubules at the interface of an artificial interstitium after 13 days. (a) Whole mount specimens are labeled by SBA. (b) An individual specimen shows 100 SBA-labeled tubules. (c) Immuno-label for cingulin on cryosections exhibits polarization of epithelial cells. (d) Reaction for Na/K-ATPase α5 is found at the basolateral aspect of cells. (e) Laminin γ1 is detected within the basal lamina. (f) Intense label for collagen III is present on the basal lamina and in the interstitial space between generated tubules.

Within the apical and lateral cytoplasm numerous lysosomal elements are found. The small, medium-sized and large vacuoles are filled to a various degree with electron-dense material suggesting it has been phagocytosed. Neighboring epithelial cells are in close contact to each other. TEM further demonstrates that the luminal and lateral plasma membranes are separated by a typical tight junctional belt consisting of a zonula occludens, zonula adhaerens and a desmosome. At the basal aspect of the epithelium a basal lamina is found. It exhibits a lamina rara interna, a lamina densa and an extended lamina fibroreticularis. All of these ultrastructural data reveal that a polarized and an obviously sealing epithelium is established within the generated tubules.

11. Inducing tubulogenic development

Development of SBA-labeled tubules is not obtained, when the culture of renal stem/progenitor cells is performed in IMDM lacking aldosterone supplementation. In this case a disintegration of the embryonic tissue is seen. In contrast, application of aldosterone (1×10^{-7} M) in IMDM induces development of numerous SBA-labeled tubules [60].

During culture chemically defined IMDM is transported from the storage bottle via silicone tubes to the perfusion container. Since serum or albumin as transport vehicle is not present, adsorption of aldosterone to the inner surface of the transport path between the storage bottle and the perfusion container may occur. To find out the optimal bioavailability for the tubulogenic effect of aldosterone, the steroid hormone was administered in concentrations ranging from 1×10^{-10} M to 1×10^{-5} M (Fig. 7). After 13 days of culture the tissue was analyzed according the aquired SBA-label. The experiments reveal that a low dose of 1×10^{-10} M aldosterone does not stimulate the development of SBA-labeled tubules (Fig. 7a). Concentrations of 1×10^{-9} M and 1×10^{-8} M stimulate growth of SBA-labeled cells forming long rows and clusters but not structured tubules (Fig. 7b,c). In contrast, intact formation of tubules is obtained by the use of 1×10^{-7} M and 1×10^{-6} M aldosterone (Fig. 7d,e). Application of a high dose of aldosterone (1×10^{-5} M) does not better stimulate tubulogenic development than obtained with lower concentrations (Fig. 7f).

From the adult kidney it is known that not only aldosterone but also precursors of the aldosterone synthesis show an affinity to the mineralocorticoid receptor (MR). The synthesis of aldosterone starts from cholesterol, which is then metabolized over pregnenolone to progesterone, 11-deoxycorticosterone, corticosterone and 18-hydroxycorticosterone. In physiological experiments it was further shown that 11-deoxycorticosterone is as effective as aldosterone on the mineralocorticoid receptor, while corticosterone is 100 times less potent [63–66].

Perfusion culture experiments with precursors (each 1×10^{-7} M) of the aldosterone synthesis pathway revealed that cholesterol and pregelonone do not show any tubulogenic activity (Fig. 8). Administration of progesterone produces single tubules but numerous cell islet with intensive SBA-label. When 11-deoxycorticosterone is applied, only few tubules with a faint SBA-label can be detected. Administration of corticosterone does not result in the development of tubules. Instead, numerous SBA-labeled cell clusters are seen in close contact to polyester fibers. Data for 18-hydroxycorticosterone are lacking, since this substance was not commercially available. In contrast, only administration of aldosterone results in numerous and intense SBA-positive tubules exhibiting a distinct lumen and a clearly recognizable basal lamina. In consequence, performed experiments demonstrate that not the precursors of the synthesis pathway but exclusively aldosterone induces tubulogenic development.

Generation of tubules takes an unexpected long period of culture time [57]. Adding aldosterone (1×10^{-7} M) to IMDM first reaction in form of slight SBA-label is detected on day 2. The binding of SBA starts

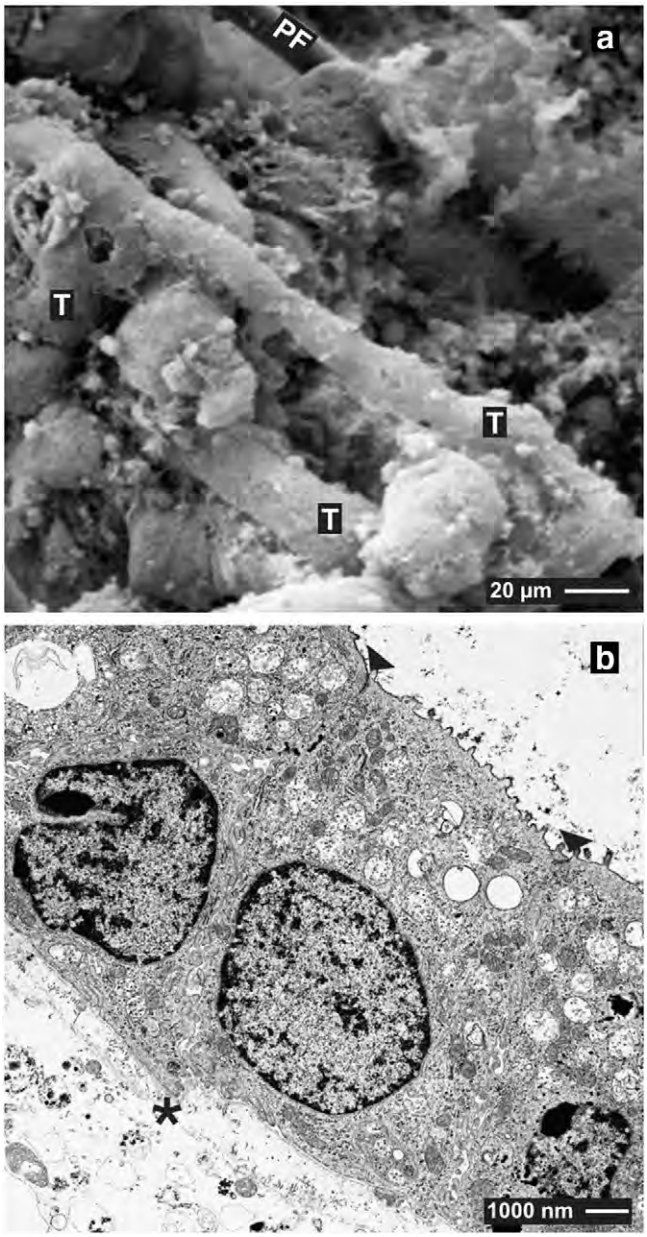

Fig. 6. Electron microscopical view to renal tubules generated for 13 days at the interface of an artificial interstitium. (a) Scanning electron microscopy demonstrates tubules (T) in the vicinity of polyester fibers (PF). On the surface of tubules single interstitial cells and fibers of extracellular matrix are observed. (b) Transmission electron microscopy demonstrates that generated tubules contain a polarized epithelium. Between the apical and lateral plasma membrane a junctional complex is developed (arrow head). At the basal side a basal lamina (asterisk) is found.

on single cells with a punctuate pattern. It increases predominantly at the luminal plasma membrane of cells that form tubules within days 3 and 5. Finally, the label is found within the whole cytoplasm of tubule cells by day 8 to 10. The intense SBA-label is stabilized between day 11 and 13 remaining then constant for weeks.

Immunohistochemical experiments of MR localization in isolated embryonic tissue failed, although a set of different antibodies (anti-MR 6D587, anti-MCR NR3C2, MCR 4i342, MCR N-17, MCR C-19, anti-idiotypic MCR H10E4C9F, anti-MRN 4E4, MRN 12A7, MRN 15D11, MRN2 2B7, anti-MRN3 3F10, anti-rMR1-18 6G1, anti-rMR365 4D6, anti-rMR1-18 1D5) was tested. To obtain information about molecular features of MR protein, SDS-electrophoresis followed by western blotting was performed [67]. Most interestingly, antibody anti-rMR1-18 1D5 revealed a distinct reaction with isolated embryonic renal tissue. One band corresponds to a 96 kDa protein, while the other revealed 88 kDa. For control, recombinant MR protein was recognized as a 96 kDa band. In consequence, the mineralocorticoid receptor could be identified for the first time in embryonic renal tissue containing stem/progenitor cells.

12. Antagonizing the action of aldosterone

Further it had to be elaborated if the tubulogenic effect of aldosterone is mediated via the mineralocorticoid receptor (MR). In these experiments aldosterone was administered in combination with antagonists such as spironolactone and canrenoate (Fig. 9) [67].

For control, intense tubule formation is observed after aldosterone (1×10^{-7} M) administration (Fig. 9a). Application of a low dose of spironolactone (1×10^{-7} M) in the presence of aldosterone (1×10^{-7} M) does not affect the development of SBA-labeled tubules. However, use of a higher concentration of spironolactone (1×10^{-5} M) demonstrates an inhibitory effect leading to a switch of development. The number of structured tubules is reduced and SBA-labeled cells start to form extended cell clusters. Presence of 1×10^{-4} M spironolactone in aldosterone-containing medium completely prevents the development of SBA-labeled tubules (Fig. 9b).

Canrenoate exhibits a similiar inhibitory profile on the tubulogenic action of aldosterone as it is observed with spironolactone. Application of 1×10^{-7} M canrenoate in the aldosterone-containing medium does not disturb the development of tubules, while administration of 1×10^{-6} M and 1×10^{-5} M canrenoate drastically reduces SBA-labeled tubules. The use of 1×10^{-4} M canrenoate results in a complete lack of SBA-labeled cells and tubules (Fig. 9c).

In consequence, the simultaneous administration of aldosterone in combination with spironolactone or canrenoate demonstrates that the tubulogenic effect is antagonized in a dose dependent manner. The result additionally reveals that the tubulogenic effect of aldosterone is mediated via the mineralocorticoid receptor.

13. Interfering the tubulogenic signal

Earlier experiments demonstrated that MR is not randomly distributed within the cytoplasm of the target cell but stays in close molecular contact with heat shock proteins (hsp) [68]. To obtain better insights in the molecular signaling renal stem/progenitor cells were treated during culture without aldosterone (Fig. 10a,b), with aldosterone (1×10^{-7} M) (Fig. 10c,d) and in combination with substances such as geldanamycin, radicicol (Fig. 9e,f), quercetin and KNK 437 (Fig. 9g,h) that disrupt these interactions [69].

To interfere the contact between MR and hsp 90 renal stem/progenitor cells were incubated in IMDM containing geldanamycin ($3{,}6\times10^{-6}$ M) in combination with aldosterone (1×10^{-7} M) for 13 days. From geldanamycin it is known that it specifically binds to hsp 90 thereby blocking the ATP-binding site due to its higher affinity compared to ATP [70]. In this series of experiments structured tubules are not found. Instead numerous SBA-labeled cells are localized in extended clusters.

Furtheron, radicicol is a macrocyclic antifungal substance that binds in the same way as geldanamycin hindering ATP-dependent conformational changes that are required for cytoplasmic interactions between target proteins such as MR [71]. Treatment of renal stem/progenitor cells with radicicol (1×10^{-6} M) in combination with aldosterone (1×10^{-7} M) produces only few structured tubules, instead numerous SBA-labeled cells in form of extended clusters were detected (Fig. 10f). In consequence, experiments with geldanamycin and radicicol show that the tubulogenic pathway of aldosterone can be blocked by interfering the contact between MR and hsp 90 [72].

Disturbing the tubulogenic signal between MR and hsp 70 by quercetin (2×10^{-4} M) produces numerous SBA-labeled cells within

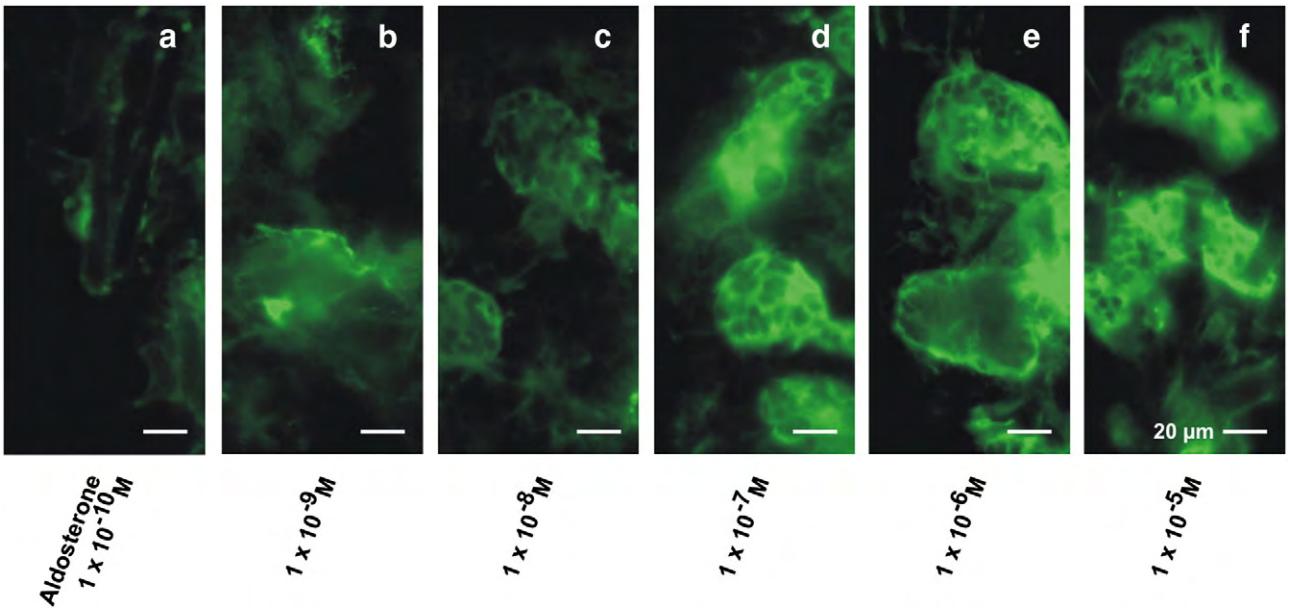

Fig. 7. Tubulogenic reaction of aldosterone after a 13 days culture period is depending on applied concentration. Control without aldosterone administration lacks SBA-labeled tubules. (a) A low dose of 1×10^{-10} M aldosterone stimulates single cells. (b) Concentrations of 1×10^{-9} M and (c) 1×10^{-8} M stimulate growth of SBA-labeled cells forming long rows and clusters but not structured tubules. Intact formation of tubules is obtained by the use of (d) 1×10^{-7} M and (e) 1×10^{-6} M aldosterone. (f) Application of 1×10^{-5} M does not further increase the development.

extended cluster formations, while only minimal development of tubules can be detected. KNK 437 is a benzylidene lactam molecule that inhibits heat shock factor activity resulting in a decreased expression of heat shock proteins, thereby interfering indirectly with MR [73]. Culture experiments with KNK 437 (1×10^{-4} M) in combination with aldosterone demonstrate that tubules are not developed, but numerous SBA-labeled cells are localized within extended cell clusters (Fig. 10h). In consequence, presented experiments illustrate that the tubulogenic signal of aldosterone is mediated via MR and an intact binding with hsp 70 [74].

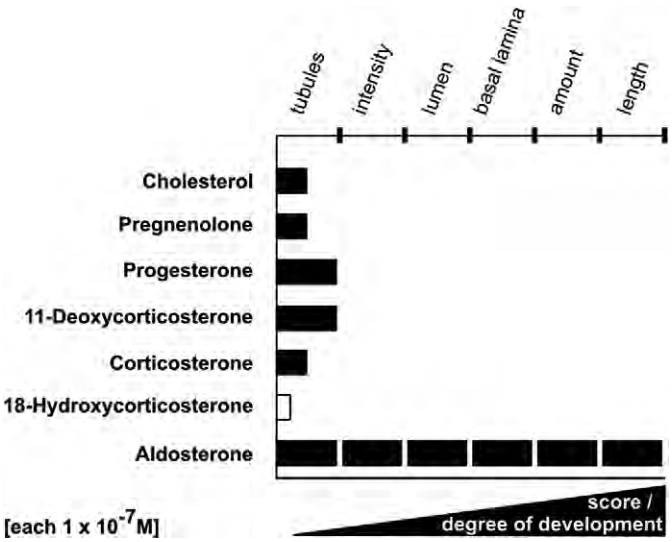

Fig. 8. Precursors of the aldosterone synthesis pathway do not stimulate development of SBA-labeled tubules. Administration of cholesterol and pregnenolone lacks tubulogenic activity, while progesterone produces few labeled cell islets. When 11-deoxycorticosterone is applied, only few tubules with a faint SBA-label can be seen. Administration of corticosterone induces numerous SBA-labeled cell clusters instead of tubules. Data for 18-hydroxycorticosterone are lacking, since this substance is not commercially available. Administration of aldosterone results in numerous SBA-positive tubules exhibiting a distinct lumen and a clearly recognizable basal lamina after 13 days of culture.

14. Featuring cell islets, cell clusters and tubules

Presented culture experiments exhibit that administration of aldosterone leads via MR binding and an intact contact with hsp 90 and 70 to the formation of numerous SBA-labeled tubules. However, one may assume that this action can be mimicked by other steroidal hormones showing certain affinity to the mineralocorticoid receptor (MR). However, application of a glucocorticoids such as 11-deoxycorticosterone (1×10^{-7} M) generates only few SBA-labeled cell islets but is not inducing the formation of numerous tubules. Administration of corticosterone (1×10^{-7} M) or dexamethasone (1×10^{-7} M) produces extended SBA-labeled cell clusters but formation of tubules is missing. These results point out that the tubulogenic action of aldosterone is a specific reaction on MR, which cannot be mimicked by mentioned glucocorticoids.

Thus, treatment of renal stem/progenitor cells by glucocorticoids does not result in the development of tubules but produces numerous SBA-labeled cell islets or extended cell clusters. To obtain more information about this phenomenon further steroidal substances were tested. Running experiments clearly exhibit four different reactions on cultured renal stem/progenitor cells. For example, cholesterol and 17-α-hydroxyprogesterone (each 1×10^{-7} M) do not reveal any recognizable effect on renal stem/progenitor cell development (Fig. 11a). In contrast, formation of SBA-labeled cell islets is found after treatment with progesterone, 11-deoxycorticosterone, dihydrotestosterone and pregnenolone (each 1×10^{-7} M) (Fig. 11b). Extensive SBA-labeled cell clusters are observed after application of testosterone, 17-β-estradiol, dexamethasone and corticosterone (each 1×10^{-7} M) (Fig. 11c). However, only the administration of aldosterone (1×10^{-7} M) leads to the formation of SBA-labeled tubules preventing formation of cell islets and clusters (Fig. 11d). Up to date no explantation can be given, why intact binding of aldosterone to MR induces development of tubules, while more or less unspecific stimulation leads to cell islets and clusters.

Summing up, the presently shown experiments demonstrate that a variety of substances has to be tested in future using renal tubules in perfusion culture as a very sensitive indicator for specific or unspecific development. Furtheron, after implantation into an organism renal stem/progenitor cells are not only exposed to aldosterone but to a

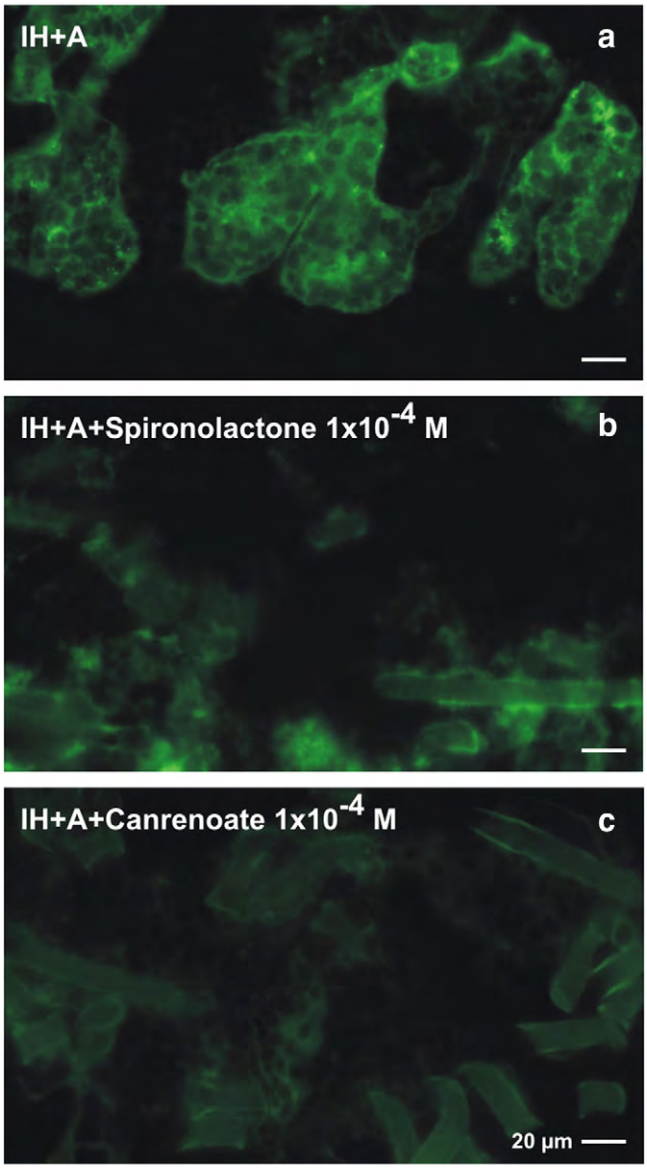

Fig. 9. Interfering the tubulogenic effect of aldosterone on the mineralocorticoid receptor. (a) Numerous tubules are observed after application of aldosterone (1×10^{-7} M). (b) Application of 1×10^{-4} M spironolactone or (c) 1×10^{-4} M canrenoate in the presence of aldosterone (1×10^{-7} M) completely inhibits the development of tubules.

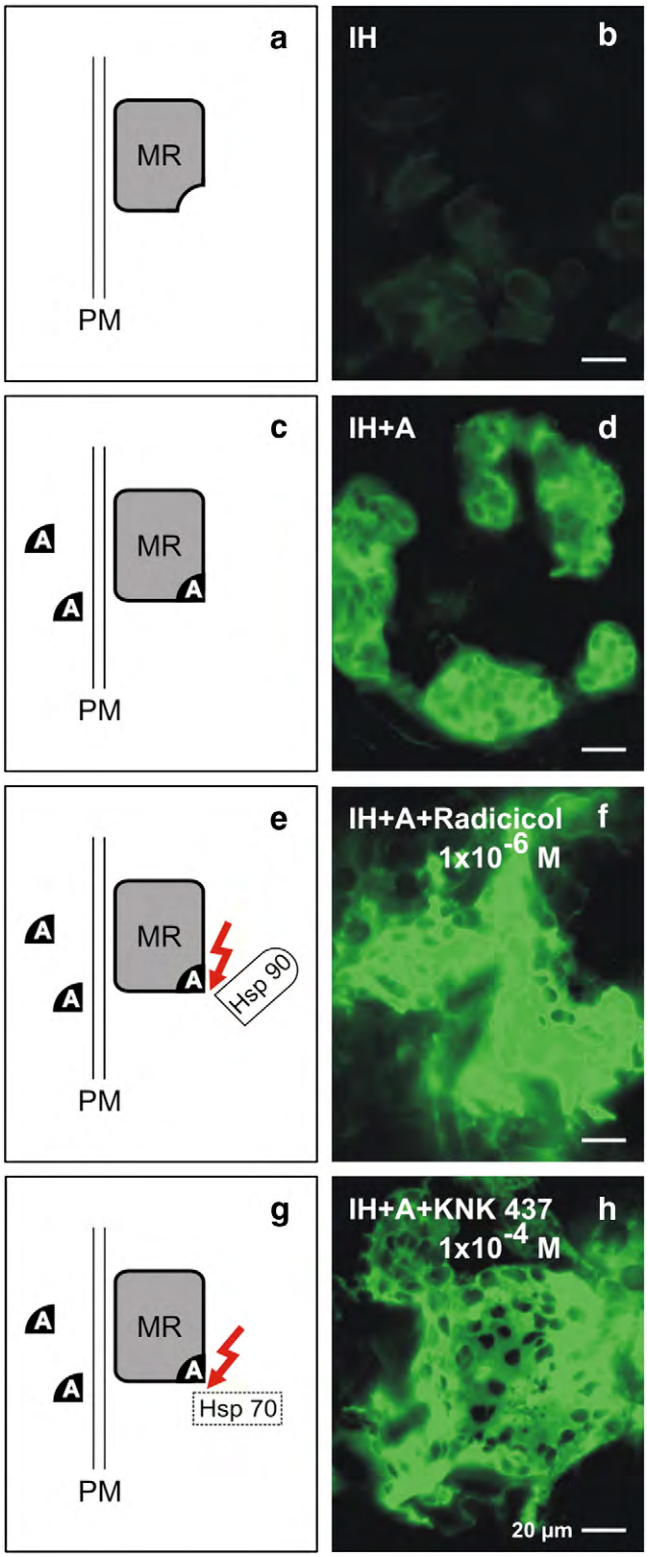

Fig. 10. Interfering the tubulogenic signal of aldosterone between the mineralocorticoid receptor and heat shock proteins (hsp). (a, b) Tubulogenic effect is missing without aldosterone treatment. (c, d) Intense formation of SBA-labeled tubules is observed after aldosterone (1×10^{-7} M) administration. Generation of SBA-labeled tubules is lacking, when aldosterone (1×10^{-7} M) is administered in combination with (e, f) radicicol (1×10^{-6} M) or (g, h) KNK 437 (1×10^{-4} M). Instead intensively labeled cell clusters are found. A – aldosterone, MR – mineralocorticoid receptor, PM – plasma membrane.

variety of other metabolites, hormones and growth factors contained in the circulating blood and interstitial fluid. On the one hand this natural cocktail of substances may exhibit beneficial effects resulting in an optimal renewal of parenchyme. On the other hand it may have an adverse effect promoting malformations or even an increase in the progress of degeneration. For that reason intense research is needed to learn about molecular steering of regeneration.

15. Supporting intact integration

Keeping a future therapeutic application in mind both the stem/progenitor cell implantation and the integration into a diseased environment need special attention. First of all, stem/progenitor cells have to be applied so that they are concentrated at the site of organ regeneration. Although frequently performed we do not favorize the concept to administer stem/progenitor cells via the vessel system by infusion [75–78]. However, infused cells are retained in the lumen of capillaries and are not concentrated at the site of parenchyma repair. Due to this reason a microsurgical subcapsular implantation appears as a better surgical procedure [79,80]. The space underneath the organ capsule is easily accessible and can be used as a depot for implanted

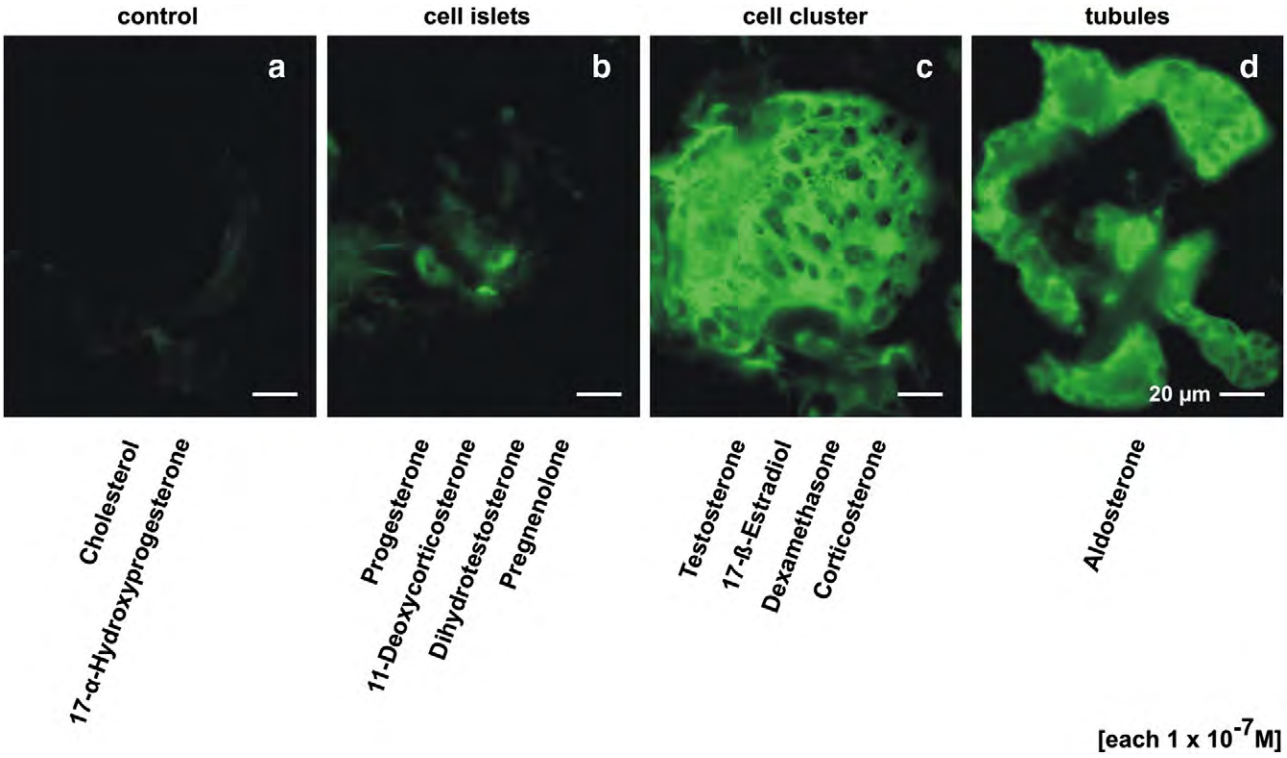

Fig. 11. Depending on steroidal substance renal stem/progenitor cells do (a) not react, (b) form cell islets, (c) intense cell clusters or (d) tubules after a 13 days perfusion culture period. (a) Cholesterol and 17-α-hydroxyprogesterone do not reveal a positive effect on renal stem/progenitor cell development. (b) Formation of SBA-labeled cell islets is found after treatment with progesterone, 11-deoxycorticosterone, dihydrotestosterone and pregnenolone. (c) Extensive SBA-labeled cell clusters containing a rough surface are observed after application of testosterone, 17-β-estradiol, dexamethasone and corticosterone. (d) Intense tubule formation is observed after aldosterone application.

stem/progenitor cells. Performing this technique the site of implantation on the organ surface and the amount of administered stem/progenitor cells can be individually adjusted. Previously performed successful implantation of insuline secreting cells illustrates a perfect survival in the subcapsular pouch of the kidney [81–83].

Furtheron, a subcapsular implantation appears also from the developmental point of view as a most promising technique for the implantation of stem/progenitor cells [84–86]. Starting from the organ anlage nephrons are only induced in the outer cortex. Step by step the kidney grows along a medullary-cortical axis. This expansion is pioneered by reciprocal interactions between the collecting duct ampulla and the surrounding nephrogenic mesenchyme. Thus, also the last generation of nephrons is induced in the outer cortex just underneath the organ capsule. When the organ is reached its final size, molecular mechanisms must be up-regulated in the outer cortex that inhibit the further induction of nephrons. Up to date sound literature about this regulation is lacking. In consequence mechanisms exist that make renal stem/progenitor cells silent in the last steps of organ growth [87]. In consequence, the idea is that the zone of the outer cortex can be reactivated by a site-specific implantation of stem/progenitor cells. For this specific application stem/progenitor cells have to be immobilized in a polyester fleece (Fig. 3c) to stick them without damage in the pouch beyond the organ capsule (Fig. 12a).

Moreover, the illustrated sandwich set-up configuration (Fig. 3c) keeps containing stem/progenitor cells in position during culture and transport by forceps. The elastic properties support the surgical handling when the implant is sticked in the pouch beyond the organ capsule. In addition, the implant exhibits the necessary stability to protect the containing stem/progenitor cells at the harmful site of implantation. The surrounding fleece equalizes mechanical and hydraulic pressure occuring between the organ capsule and the outer cortex of parenchyma. Fibers of the fleece simulate the extracellular matrix so that regeneration of tubules can start (Fig. 12b). During these first steps of development the space between the fibers supports optimal nutrition and exchange of respiratory gas.

The implantation site beyond the capsule is avascular. However, for a perfect regeneration an ingrowth of capillaries is necessary [88]. For that reason an implanted fleece provides a perfect interaction with the surrounding parenchyma of the diseased kidney so that ingrowth of capillaries can start in parallel to the initiation of regeneration (Fig. 12c).

Finally, an implanted fleece must be fully biocompatible not only for the containing stem/progenitor cells and the invading capillaries but also for the surrounding diseased parenchyma (Fig. 12d). Only a perfect concert of interactions between the implanted fleece, the choosen stem/progenitor cells, the invading capillaries and diseased parenchyma will promote the complex process of stem/progenitor cell development resulting finally in a spatial organization of regenerated parenchyma.

16. Improving spatial development

The presented experiments reveal for the first time that it is possible to register tubule formation under controlled culture conditions. Since coating by extracellular matrix proteins was avoided, spatial organization of tubules can be critically analyzed in combination with a selected biomaterial. An essential finding in this coherence was that perfect tubule formation only occurs, when stem/progenitor cells are cultured between layers of an I7 polyester fleece. Applying this innovative culture approach cells do not migrate into the space between the polyester fibers, but stay at the interface between the covering fleece layers (Fig. 13a,b). This specific arrangement promotes on the one hand the spatial development of tubules. However, on the other hand the application of an I7 fleece appears detrimental for a subcapsular implantation in the kidney due to its thickness and the extended dead space volume.

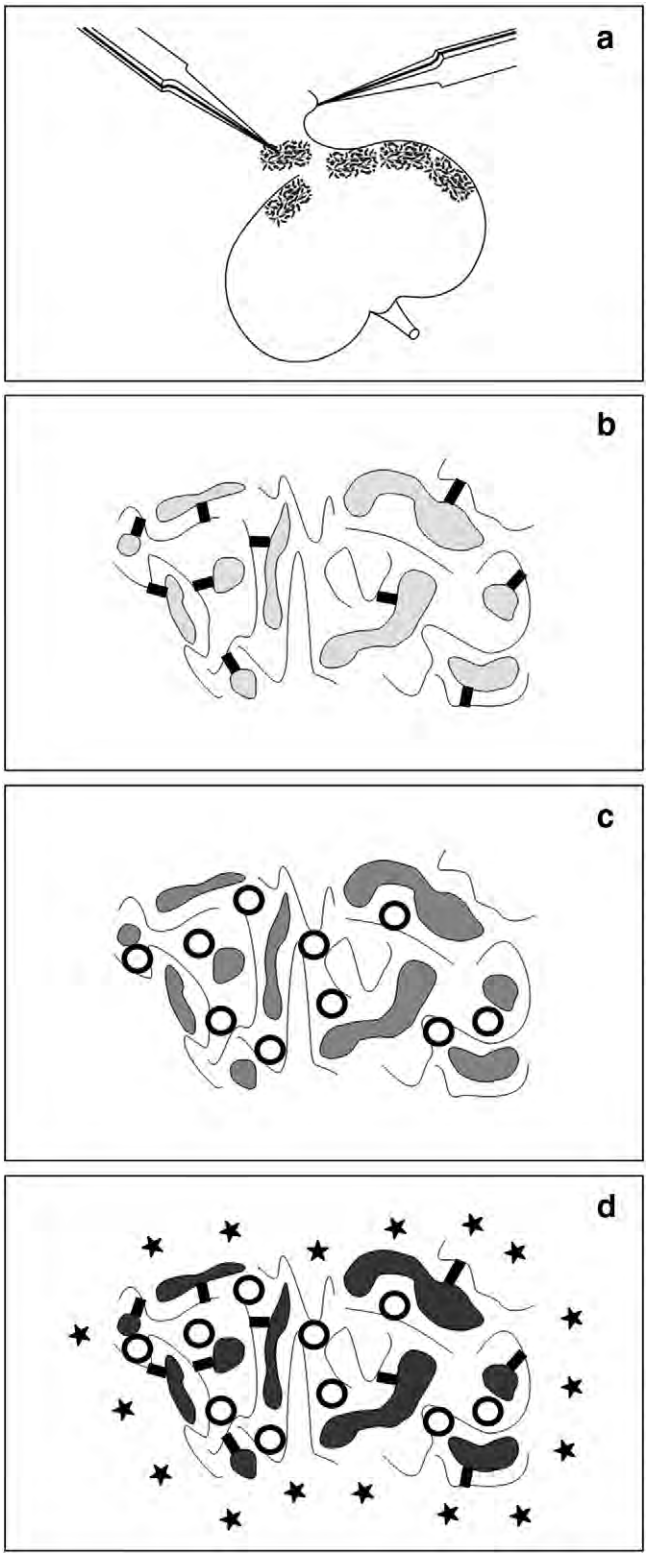

Fig. 12. Stem/progenitor cells within an artificial interstitium used as a subcapsular implant. (a) Sandwich set-ups can be implanted in the subcapsular space. (b) Development depends on a positive interaction with fleece fibers. (c) Optimal development needs ingrowth of capillaries. (d) Functional development is highly dependent on the neighboring parenchyma.

Following a subcapsular implantation the stem/progenitor cells have to be applied in a concentrated manner so that extended dead space volume is avoided. This could be reached by piling basic sandwich set-ups containing stem/progenitor cells (Fig. 12a) [89]. When layers of an I7 polyester fleece are piled, layers of stem/progenitor cells are separated by 0.6 mm. For an implantation the dead space volume is too extended in relation to containing stem/progenitor cells. To concentrate more efficiently stem/progenitor cells the dead space volume of the fleece has to be minimized by applying polyester fleeces with a decreased thickness. That was the reason to investigate different kinds of polyester fleeces showing a minimal thickness.

Selecting a variety of fleece materials finally Posi-4, Posi-5, Posi-6 and Posi-7 polyester fleeces (Positech, Hallwil, Suisse) were selected. The fleeces exhibit a decreased thickness between 0.13 and 0.18 mm [90]. The first interesting finding with applied Posi-4, Posi-5, Posi-6 and Posi-7 polyester fleeces was that they do not reveal any toxic influence, when renal stem/progenitor cells are kept in contact with them during a perfusion culture period of 13 days. After administration of aldosterone in all of the cases an intense pattern of SBA-labeled tubules can be detected as earlier found with an I7 fleece. Most interestingly, each of applied Posi polyester fleeces produces its individual spatial growth pattern. For the first time it is observed that the space beween the Posi-4 polyester fibers is accepted by renal stem/progenitor cells for the development of tubules (Fig. 13c,d). Like in a woven textile formation of numerous tubules is detected in the space between the fleece fibers. In addition, the growth of tubules between the fleece fibers reduces significantly the dead space volume. Thus, using Posi fleeces (Fig. 13c,d) the portion of implanted stem/progenitor cells can be increased, while the volume of polyester fleece is minimized as compared to earlier used I7 fleece (Fig. 13a,b). For the first time a fleece for a subcapsular implantation is in sight, which concentrates renal stem/progenitor cells and which appears suitable to promote optimally the spatial regeneration of renal tubules.

17. Stimulating formation by polymers and drug delivery

A successful candidate of fleece used for a subcapsular implantation in the kidney must show full biocompatibility not only for the containing stem/progenitor cells but also for the surrounding diseased parenchyma (Fig. 12d). Beside integration it has to promote a complex developmental process leading from embryonic cells to functional parenchyma. A fleece appears further optimal, when the containing material exhibits biodegradable features [91–94]. As the new parenchyma forms the implanted fleece disappears without disturbing the proceeding spatial regeneration. During ongoing development the implanted fleece must be non-toxic, non-immunogenic, non-attractive and non-stimulatory for inflammatory cells. However, up to date these features could not be tested in performed experiments. Therefore it is imaginable that beside I7 and Posi fleeces a spectrum of other synthetic polymers may exist supporting still better the spatial development of parenchyma during renal repair [95].

Not only the described endoskeletonal tasks of an implanted fleece are of fundamental importance. The spatial development of parenchyma was stimulated in present experiments by the administration of aldosterone (Figs. 7 and 8). It is imaginable that beside aldosterone a variety of morphogens, growth factors or hormones exists that can be used to promote the process of regeneration. In parallel inflammation may be suppressed locally by a variety of pharmaceuticals. For that reason an implanted fleece material may contain drug delivery systems designed to release active agents supporting the spatial development and preventing malformations, inflammation and infections [96]. It is imaginable that the course of development is influenced by molecules containing active motives that are integrated in the implanted fleece fibers. Their liberation may derive from simple depot delivery systems such as integrated microspheres, beads or minipellets. The advantage of such depots is the release of mentioned factors into the local environment for a limited period of time promoting spatial development of parenchyma at the site of need

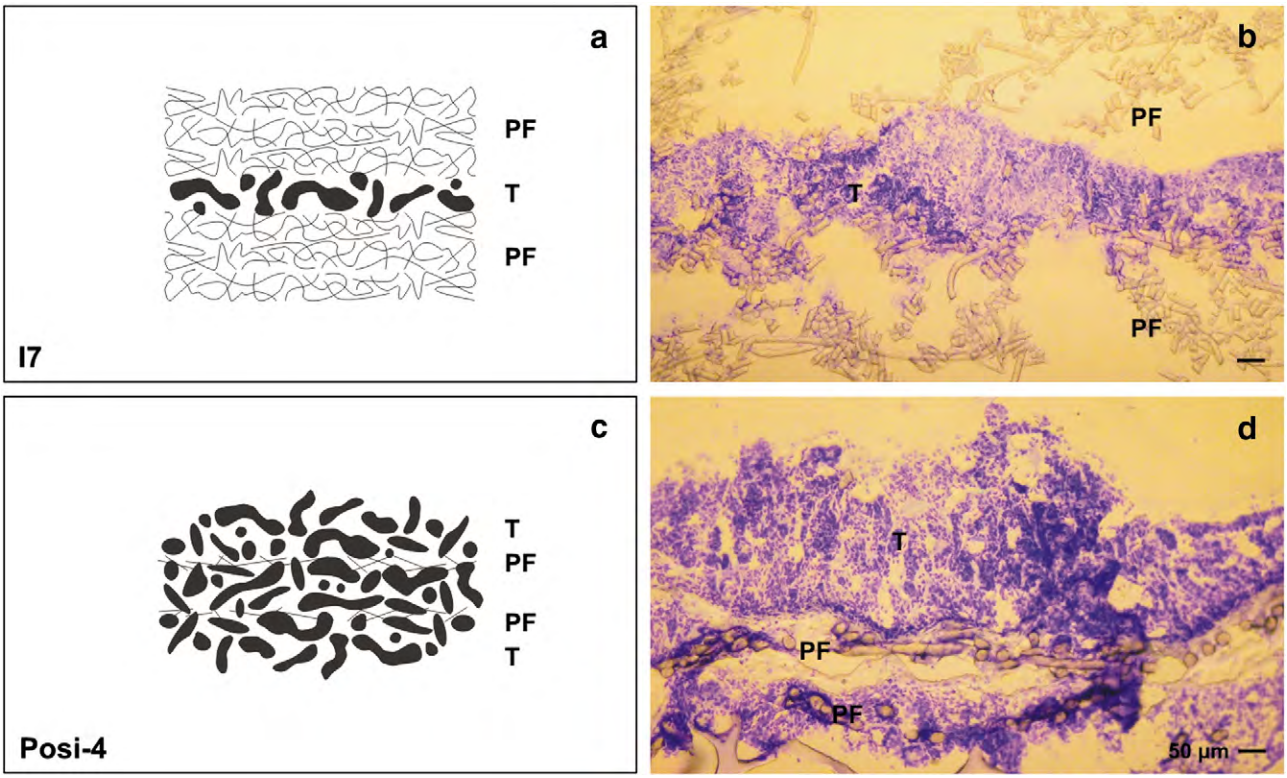

Fig. 13. Generation of tubules at an artificial interstitium. (a, b) Artificial interstitium made of an I7 polyester fleece (PF). (a) Schematic illustration shows growth of tubules between two fleece layers. (b) Toluidine blue-stain of a cryosection demonstrates numerous tubules (T) growing at the interface between I7 fleeces of polyester. (c, d) Artificial interstitium made of a Posi-4 polyester fleece. (c) Schematic illustration depicts growth of tubules within and around the Posi-4 fleece. (d) Toluidine blue-stain of a cryosection demonstrates numerous tubules (T) growing within and around a Posi-4 fleece.

[97]. Otherwise it has to be considered that the regeneration of parenchyme happens in an inflammatory environment. For that reason mentioned delivery systems may be used to liberate at the site of need drugs suppressing inflammation. Release of such substances may occur only in defined phases of development so that they do not harm the organisation of regenerating tissue.

Most interesting systems are biologically inspired materials that release growth factors by cellular demand as it was for example successfully shown with polyethylenglycol (PEG) hydrogels [45]. In consequence, the future challenge in the repair of kidney is therefore the provision of factors supporting development at the right place of need and at a sustained release rate for a positive parenchyma response [98–100]. Most important, extensive research is needed so that in future acute and chronic renal failure may be treated by proposed innovative therapeutic concepts.

18. Summing up

Regeneration of parenchyme in acute and chronic renal failure is limited. To investigate basic mechanisms of renal tubule regeneration by the help of renal stem/progenitor cells an innovative in vitro approach was established. To support spatial development renal stem/progenitor cells are kept at the interface of an artificial interstitium made by a I7 polyester fleece covering the upper and lower sides in a basic sandwich set-up during long term perfusion culture. This method replaces coating by extracellular matrix proteins and makes experiments in chemically defined medium possible.

The presented experiments illuminate that aldosterone exhibits a unique tubulogenic influence on renal stem/progenitor cells. Omittance of the hormone leads to tissue disintegration, while presence of aldosterone results in the development of numerous SBA-labeled tubules. It is demonstrated that aldosterone acts in a concentration-dependent fashion. Antagonists such as spironolactone and canrenoate prevent the development of tubules indicating that the tubulogenic effect of aldosterone is mediated via stimulation of the mineralocorticoid receptor (MR). The signal is interrupted, when the molecular contact site between the mineralocorticoid receptor and neighboring heat shock proteins 90 and 70 is disturbed using geldanamycin respectively quercetin.

Most interestingly, when other steroidal hormones with affinity to the glucocorticoid (GR) and mineralocorticoid receptor such as 11-deoxycorticosterone, corticosterone and dexamethasone are applied, formation of tubules is lacking. Instead, SBA-labeled cell islets or extensive cell clusters are developing. Up to date it is not known, why only the binding of aldosterone promotes the development of tubules and why binding of other steroid hormones prevents it. For that reason possible interactions between the glucocorticoid and mineralocorticoid receptor are in the focus of actual research. It is imaginable that beside aldosterone also a variety of other hormones, growth factors and morphogens exist that influence regeneration of intact renal parenchyma.

Application of different kinds of polyester fleeces demonstrates that the developmental pattern of SBA-labeled tubules depends on the selected fleece. Growth of tubules can occur at the interface between fleece layers (I7) but also in the space between the fleece fibers (Posi). The integration of tubules between the fleece fibers reduces significantly dead space volume so that the portion of parenchyma can be increased, while the portion of polyester fleece is minimized. In consequence, for the first time a biomaterial for implantation is in sight, which exhibits ideal features for the spatial regeneration of renal parenchyma. Furtheron, it is imaginable that the regeneration of parenchyma is promoted by newly developed pharmaceuticals, hormones, growth factors or morphogens that are integrated in the applied fleece fiber material. These substances could

be released by sophisticated drug delivery systems at the right time and exclusively at the site of occuring regeneration.

References

[1] P. Romagnani, Toward the identification of a "renopoietic system"? Stem Cells 9 (2009) 2247–2253.
[2] A.W. Duncan, C. Dorrell, M. Grompe, Stem cells and liver regeneration, Gastroenterology 2 (2009) 466–481.
[3] P. Sancho-Bru, M. Najimi, M. Caruso, K. Pauwelyn, T. Cantz, S. Forbes, T. Roskams, M. Ott, U. Gehling, E. Sokal, C.M. Verfaillie, M. Muraca, Stem and progenitor cells for liver repopulation: can we standardise the process from bench to bedside? Gut 4 (2009) 594–603.
[4] P. Chhabra, K.L. Brayman, The use of stem cells in kidney disease, Curr Opin Organ Transpl 1 (2009) 72–78.
[5] B. Bussolati, P.V. Hauser, R. Carvalhosa, G. Camussi, Contribution of stem cells to kidney repair, Curr Stem Cell Res Ther 1 (2009) 2–8.
[6] B.D. Humphreys, J.D. Duffield, J.V. Bonventre, Renal stem cells in recovery from acute kidney injury, Minerva Urol. Nefrol. 1 (2006) 13–21.
[7] M.H. Little, F.K. Rae, Review article: Potential cellular therapies for renal disease: can we translate results from animal studies to the human condition? Nephrology (Carlton, Vic.) 6 (2009) 544–553.
[8] C. Hopkins, J. Li, F. Rae, M.H. Little, Stem cell options for kidney disease, J. Pathol. 2 (2009) 265–281.
[9] A.L. Cochrane, M.M. Kett, C.S. Samuel, N.V. Campanale, W.P. Anderson, D.A. Hume, M.H. Little, J.F. Bertram, S.D. Ricardo, Renal structural and functional repair in a mouse model of reversal of ureteral obstruction, J. Am. Soc. Nephrol. 12 (2005) 3623–3630.
[10] R.L. Chevalier, M.S. Forbes, B.A. Thornhill, Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy, Kidney Int. 11 (2009) 1145–1152.
[11] N.I. Alcalay, G.B. Vanden Heuvel, Regulation of cell proliferation and differentiation in the kidney, Front. Biosci. (2009) 4978–4991.
[12] E.A. Ross, M.J. Williams, T. Hamazaki, N. Terada, W.L. Clapp, C. Adin, G.W. Ellison, M. Jorgensen, C.D. Batich, Embryonic Stem Cells Proliferate and Differentiate when Seeded into Kidney Scaffolds, J. Am. Soc. Nephrol. 20 (11) (2009) 2338–2347.
[13] S. Ishibe, L.G. Cantley, Epithelial-mesenchymal-epithelial cycling in kidney repair, Curr. Opin. Nephrol. Hypertens. 4 (2008) 379–385.
[14] R. Masereeuw, Contribution of bone marrow-derived cells in renal repair after acute kidney injury, Minerva Urol. Nefrol. 4 (2009) 373–384.
[15] W. Thomas, R. Dooley, B.J. Harvey, Aldosterone as a renal growth factor, Steroids (2009) [Electronic publication ahead of print], doi:10.1016/j.steroids.2009.09.008.
[16] H. Zhang, F. Tasnim, J.Y. Ying, D. Zink, The impact of extracellular matrix coatings on the performance of human renal cells applied in bioartificial kidneys, Biomaterials 15 (2009) 2899–2911.
[17] M.R. Hammerman, Windows of opportunity for organogenesis, Transpl. Immunol. 1 (2005) 1–8.
[18] M.R. Hammerman, Growing kidneys, Curr. Opin. Nephrol. Hypertens. 1 (2001) 13–17.
[19] Z. Saifudeen, S. Dipp, J. Stefkova, X. Yao, S. Lookabaugh, S.S. El-Dahr, p53 Regulates Metanephric Development, J. Am. Soc. Nephrol. 20 (11) (2009) 2328–2337.
[20] W. Kriz, Ontogenetic development of the filtration barrier, Nephron Exp. Nephrol. 2 (2007) e44–e50.
[21] A. Marlier, K.M. Schmidt-Ott, A.-R. Gallagher, J. Barasch, A. Karihaloo, Vegf as an epithelial cell morphogen modulates branching morphogenesis of embryonic kidney by directly acting on the ureteric bud, Mech. Dev. 3–4 (2009) 91–98.
[22] H.J. Han, W.J. Sigurdson, P.A. Nickerson, M. Taub, Both mitogen activated protein kinase and the mammalian target of rapamycin modulate the development of functional renal proximal tubules in matrigel, J. Cell. Sci. Pt 9 (2004) 1821–1833.
[23] M.A. Schlüter, B. Margolis, Apical lumen formation in renal epithelia, J. Am. Soc. Nephrol. 7 (2009) 1444–1452.
[24] K. Georgas, B. Rumballe, M.T. Valerius, H.S. Chiu, R.D. Thiagarajan, E. Lesieur, B.J. Aronow, E.W. Brunskill, A.N. Combes, D. Tang, D. Taylor, S.M. Grimmond, S.S. Potter, A.P. McMahon, M.H. Little, Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment, Dev. Biol. 332 (2) (2009) 273–286.
[25] D. Raciti, L. Reggiani, L. Geffers, Q. Jiang, F. Bacchion, A.E. Subrizi, D. Clements, C. Tindal, D.R. Davidson, B. Kaissling, A.W. Brändli, Organization of the pronephric kidney revealed by large-scale gene expression mapping, Genome Biol. 5 (2008) R84.
[26] B.L.M. Hogan, P.A. Kolodziej, Organogenesis: molecular mechanisms of tubulogenesis, Nat. Rev. Genet. 7 (2002) 513–523.
[27] B. Lubarsky, M.A. Krasnow, Tube morphogenesis: making and shaping biological tubes, Cell 1 (2003) 19–28.
[28] E. Rosines, R.V. Sampogna, K. Johkura, D.A. Vaughn, Y. Choi, H. Sakurai, M.M. Shah, S.K. Nigam, Staged in vitro reconstitution and implantation of engineered rat kidney tissue, Proc. Natl. Acad. Sci. U. S. A. 52 (2007) 20938–20943.
[29] M. Guarino, A. Tosoni, M. Nebuloni, Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition, Hum. Pathol. 10 (2009) 1365–1376.
[30] R.P. Woroniecki, H.W. Schnaper, Progression of glomerular and tubular disease in pediatrics, Semin. Nephrol. 4 (2009) 412–424.
[31] C.M. Karner, R. Chirumamilla, S. Aoki, P. Igarashi, J.B. Wallingford, T.J. Carroll, Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis, Nat. Genet. 7 (2009) 793–799.
[32] E.J. Sharples, Acute kidney injury: stimulation of repair, Curr. Opin. Crit. Care 6 (2007) 652–655.
[33] S.R. Ash, F.E. Cuppage, M.E. Hoses, E.E. Selkurt, Culture of isolated renal tubules: a method of assessing viability of normal and damaged cells, Kidney Int. 1 (1975) 55–60.
[34] M. Horster, Primary culture of mammalian nephron epithelia: requirements for cell outgrowth and proliferation from defined explanted nephron segments, Pflugers Arch. 3 (1979) 209–215.
[35] P.C. Baer, H. Geiger, Human renal cells from the thick ascending limb and early distal tubule: characterization of primary isolated and cultured cells by reverse transcription polymerase chain reaction, Nephrology (Carlton, Vic.) 4 (2008) 316–321.
[36] D.A. Vesey, W. Qi, X. Chen, C.A. Pollock, D.W. Johnson, Isolation and primary culture of human proximal tubule cells, Methods Mol. Biol. (2009) 19–24.
[37] C. Leimeister, A. Bach, A.S. Woolf, M. Gessler, Screen for genes regulated during early kidney morphogenesis, Dev. Genet. 3–4 (1999) 273–283.
[38] K.T. Bush, D.A. Vaughn, X. Li, M.G. Rosenfeld, D.W. Rose, S.A. Mendoza, S.K. Nigam, Development and differentiation of the ureteric bud into the ureter in the absence of a kidney collecting system, Dev. Biol. 2 (2006) 571–584.
[39] S. Terryn, F. Jouret, F. Vandenabeele, I. Smolders, M. Moreels, O. Devuyst, P. Steels, E. van Kerkhove, A primary culture of mouse proximal tubular cells, established on collagen-coated membranes, Am. J. Physiol. Renal Physiol. 2 (2007) F476–F485.
[40] C.D.A. Brown, R. Sayer, A.S. Windass, I.S. Haslam, M.E. de Broe, P.C. D'Haese, A. Verhulst, Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling, Toxicol. Appl. Pharmacol. 3 (2008) 428–438.
[41] C.N. Inoue, N. Sunagawa, T. Morimoto, S. Ohnuma, F. Katsushima, T. Nishio, Y. Kondo, K. Iinuma, Reconstruction of tubular structures in three-dimensional collagen gel culture using proximal tubular epithelial cells voided in human urine, In Vitro Cell. Dev. Biol. Anim. 8–9 (2003) 364–367.
[42] A. Joraku, K.A. Stern, A. Atala, J.J. Yoo, In vitro generation of three-dimensional renal structures, Methods 2 (2009) 129–133.
[43] R.O. Stuart, S.K. Nigam, Development of the tubular nephron, Semin. Nephrol. 4 (1995) 315–326.
[44] N.E. Hellman, J. Spector, J. Robinson, X. Zuo, S. Saunier, C. Antignac, J.W. Tobias, J.H. Lipschutz, Matrix metalloproteinase 13 (MMP13) and tissue inhibitor of matrix metalloproteinase 1 (TIMP1), regulated by the MAPK pathway, are both necessary for Madin-Darby canine kidney tubulogenesis, J. Biol. Chem. 7 (2008) 4272–4282.
[45] I.-M. Chung, N.O. Enemchukwu, S.D. Khaja, N. Murthy, A. Mantalaris, A.J. García, Bioadhesive hydrogel microenvironments to modulate epithelial morphogenesis, Biomaterials 17 (2008) 2637–2645.
[46] M. Delous, N.E. Hellman, H.-M. Gaudé, F. Silbermann, A. Le Bivic, R. Salomon, C. Antignac, S. Saunier, Nephrocystin-1 and nephrocystin-4 are required for epithelial morphogenesis and associate with PALS1/PATJ and Par6, Hum. Mol. Genet. 18 (24) (2009) 4711–4723.
[47] S.-Y. Han, H.S. Hwang, J.S. Chae, S.-J. Yang, J.-H. Yoon, Y.I. Yeom, E.-J. Choi, CIIA induces the epithelial-mesenchymal transition and cell invasion, Biochem. Biophys. Res. Commun. 3 (2009) 548–552.
[48] P.R. Brakeman, K.D. Liu, K. Shimizu, Y. Takai, K.E. Mostov, Nectin proteins are expressed at early stages of nephrogenesis and play a role in renal epithelial cell morphogenesis, Am. J. Physiol. Renal Physiol. 3 (2009) F564–F574.
[49] K.-X. Zhang, K.R. Ward, J.W. Schrader, Multiple aspects of the phenotype of mammary epithelial cells transformed by expression of activated M-Ras depend on an autocrine mechanism mediated by hepatocyte growth factor/scatter factor, Mol. Cancer Res. 4 (2004) 242–255.
[50] W.-C. Wei, A.K. Kopec, M.-J. Tang, Requirement of focal adhesion kinase in branching tubulogenesis, J. Biomed. Sci. (2009) 5, doi:10.1186/1423-0127-16-5.
[51] H.K. Kleinman, G.R. Martin, Matrigel: basement membrane matrix with biological activity, Semin. Cancer Biol. 5 (2005) 378–386.
[52] W.W. Minuth, R. Strehl, K. Schumacher, Tissue factory: conceptual design of a modular system for the in vitro generation of functional tissues, Tissue Eng. 1–2 (2004) 285–294.
[53] W.W. Minuth, L. Sorokin, K. Schumacher, Generation of renal tubules at the interface of an artificial interstitium, Cell. Physiol. Biochem. 4-6 (2004) 387–394.
[54] W.W. Minuth, Neonatal rabbit kidney cortex in culture as tool for the study of collecting duct formation and nephron differentiation, Differentiation 1 (1987) 12–22.
[55] K.J. Reidy, N.D. Rosenblum, Cell and molecular biology of kidney development, Semin. Nephrol. 4 (2009) 321–337.
[56] R. Strehl, V. Trautner, S. Kloth, W.W. Minuth, Existence of a dense reticular meshwork surrounding the nephron inducer in neonatal rabbit kidney, Cell Tissue Res. 3 (1999) 539–548.
[57] S. Heber, L. Denk, K. Hu, W.W. Minuth, Modulating the development of renal tubules growing in serum-free culture medium at an artificial interstitium, Tissue Eng. 2 (2007) 281–292.
[58] R. Strehl, K. Schumacher, W.W. Minuth, Controlled respiratory gas delivery to embryonic renal epithelial explants in perfusion culture, Tissue Eng. 10 (7/8) (2004) 1196–1203.
[59] K. Hu, L. Denk, U. de Vries, W.W. Minuth, Chemically defined medium environment for the development of renal stem cells into tubules, Biotechnol. J. 8 (2007) 992–995.

[60] W.W. Minuth, L. Denk, K. Hu, The role of polyester interstitium and aldosterone during structural development of renal tubules in serum-free medium, Biomaterials 30 (2007) 4418–4428.
[61] A. Blattmann, L. Denk, R. Strehl, H. Castrop, W.W. Minuth, The formation of pores in the basal lamina of regenerated renal tubules, Biomaterials 18 (2008) 2749–2756.
[62] W.W. Minuth, L. Denk, C. Meese, R. Rachel, A. Roessger, Ultrastructural insights in the interface between generated renal tubules and a polyester interstitium, Langmuir 8 (2009) 4621–4627.
[63] B. Escoubet, C. Coureau, M. Blot-Chabaud, J.P. Bonvalet, N. Farman, Corticosteroid receptor mRNA expression is unaffected by corticosteroids in rat kidney, heart, and colon, Am. J. Physiol. 5 (Pt 1) (1996) C1343–C1353.
[64] M.-E. Rafestin-Oblin, J. Fagart, A. Souque, C. Seguin, M. Bens, A. Vandewalle, 11beta-hydroxyprogesterone acts as a mineralocorticoid agonist in stimulating Na + absorption in mammalian principal cortical collecting duct cells, Mol. Pharmacol. 6 (2002) 1306–1313.
[65] H.-P. Gaeggeler, E. Gonzalez-Rodriguez, N.F. Jaeger, D. Loffing-Cueni, R. Norregaard, J. Loffing, J.-D. Horisberger, B.C. Rossier, Mineralocorticoid versus glucocorticoid receptor occupancy mediating aldosterone-stimulated sodium transport in a novel renal cell line, J. Am. Soc. Nephrol. 4 (2005) 878–891.
[66] A. Sturm, N. Bury, L. Dengreville, J. Fagart, G. Flouriot, M.E. Rafestin-Oblin, P. Prunet, 11-deoxycorticosterone is a potent agonist of the rainbow trout (Oncorhynchus mykiss) mineralocorticoid receptor, Endocrinology 1 (2005) 47–55.
[67] W.W. Minuth, L. Denk, K. Hu, H. Castrop, C. Gomez-Sanchez, The tubulogenic effect of aldosterone is attributed to intact binding and intracellular response of the mineralocorticoid receptor, CEJB CEJB 2 (3) (2007) 3307–3325.
[68] C.M. Bamberger, M. Wald, A.M. Bamberger, H.M. Schulte, Inhibition of mineralocorticoid and glucocorticoid receptor function by the heat shock protein 90-binding agent geldanamycin, Mol. Cell. Endocrinol. 2 (1997) 233–240.
[69] W.W. Minuth, A. Blattmann, L. Denk, H. Castrop, Mineralocorticoid Receptor, Heat Shock Proteins and Immunophilins Participate in the Transmission of the Tubulogenic Signal of Aldosterone, J. Epithel. Biol. Pharmacol. 11 (2008) 24–34.
[70] M.F. Barginear, C. van Poznak, N. Rosen, S. Modi, C.A. Hudis, D.R. Budman, The heat shock protein 90 chaperone complex: an evolving therapeutic target, Curr. Cancer Drug Targets 6 (2008) 522–532.
[71] Z. Batulan, D.M. Taylor, R.J. Aarons, S. Minotti, M.M. Doroudchi, J. Nalbantoglu, H. D. Durham, Induction of multiple heat shock proteins and neuroprotection in a primary culture model of familial amyotrophic lateral sclerosis, Neurobiol. Dis. 2 (2006) 213–225.
[72] V. Ramírez, N. Uribe, R. García-Torres, C. Castro, J. Rubio, G. Gamba, N.A. Bobadilla, Upregulation and intrarenal redistribution of heat shock proteins 90alpha and 90beta by low-sodium diet in the rat, Cell Stress Chaperones 2 (2004) 198–206.
[73] L.A. Manwell, J.J. Heikkila, Examination of KNK437- and quercetin-mediated inhibition of heat shock-induced heat shock protein gene expression in Xenopus laevis cultured cells, Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 3 (2007) 521–530.
[74] K.L. Bruner, A. Derfoul, N.M. Robertson, G. Guerriero, T. Fernandes-Alnemri, E.S. Alnemri, G. Litwack, The unliganded mineralocorticoid receptor is associated with heat shock proteins 70 and 90 and the immunophilin FKBP-52, Receptors Dignal Transduct. 2 (1997) 85–98.
[75] M. Morigi, M. Introna, B. Imberti, D. Corna, M. Abbate, C. Rota, D. Rottoli, A. Benigni, N. Perico, C. Zoja, A. Rambaldi, A. Remuzzi, G. Remuzzi, Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice, Stem Cells 8 (2008) 2075–2082.
[76] H.C. Caldas, I.M. Fernandes, F. Gerbi, A.C. Souza, M.A. Baptista, H.J. Ramalho, R.S. Kawasaki-Oyama, E.M. Goloni-Bertollo, E.C. Pavarino-Bertelli, D.M. Braile, M. Abbud-Filho, Effect of whole bone marrow cell infusion in the progression of experimental chronic renal failure, Transplant. Proc. 3 (2008) 853–855.
[77] T. Yokoo, T. Kawamura, Xenobiotic kidney organogenesis: a new avenue for renal transplantation, J. Nephrol. 3 (2009) 312–317.
[78] A.R. Chade, X. Zhu, R. Lavi, J.D. Krier, S. Pislaru, R.D. Simari, C. Napoli, A. Lerman, L.O. Lerman, Endothelial progenitor cells restore renal function in chronic experimental renovascular disease, Circulation 4 (2009) 547–557.
[79] G.J. Bakeine, A. Bertolotti, M. Latina, T. Congiu, U. Prati, L. Roveda, F. Trotta, M. Tormen, E. Di Fabrizio, G. Carlini, A. Facoetti, R. Nano, Surface properties and implantation site affect the capsular fibrotic overgrowth, J Biomed Mater Res Part A 4 (2007) 965–969.
[80] L.M. Curtis, S. Chen, B. Chen, A. Agarwal, C.A. Klug, P.W. Sanders, Contribution of intrarenal cells to cellular repair after acute kidney injury: subcapsular implantation technique, Am. J. Physiol. Renal Physiol. 1 (2008) F310–F314.
[81] D. Dufrane, M. van Steenberghe, R.-M. Goebbels, A. Saliez, Y. Guiot, P. Gianello, The influence of implantation site on the biocompatibility and survival of alginate encapsulated pig islets in rats, Biomaterials 17 (2006) 3201–3208.
[82] X. Chen, X. Zhang, F. Chen, C.S. Larson, L.-J. Wang, D.B. Kaufman, Comparative study of regenerative potential of beta cells from young and aged donor mice using a novel islet transplantation model, Transplantation 4 (2009) 496–503.
[83] G.-h. Mao, G.-a. Chen, H.-y. Bai, T.-r. Song, Y.-x. Wang, The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds seeded with islet-like cells derived from human embryonic stem cells, Biomaterials 9 (2009) 1706–1714.
[84] E.V. Lerma, Anatomic and physiologic changes of the aging kidney, Clin. Geriatr. Med. 3 (2009) 325–329.
[85] M.M. Shah, J.B. Tee, T. Meyer, C. Meyer-Schwesinger, Y. Choi, D.E. Sweeney, T.F. Gallegos, K. Johkura, E. Rosines, V. Kouznetsova, D.W. Rose, K.T. Bush, H. Sakurai, S.K. Nigam, The instructive role of metanephric mesenchyme in ureteric bud patterning, sculpting, and maturation and its potential ability to buffer ureteric bud branching defects, Am. J. Physiol. Renal Physiol. 5 (2009) F1330–F1341.
[86] J. Dötsch, C. Plank, K. Amann, J. Ingelfinger, The implications of fetal programming of glomerular number and renal function, J. Mol. Med. 9 (2009) 841–848.
[87] M.H. Little, J.F. Bertram, Is there such a thing as a renal stem cell? J. Am. Soc. Nephrol. 10 (2009) 2112–2117.
[88] F. Li, W. Li, S. Johnson, D. Ingram, M. Yoder, S. Badylak, Low-molecular-weight peptides derived from extracellular matrix as chemoattractants for primary endothelial cells, Endothelium 3–4 (2004) 199–206.
[89] W.W. Minuth, L. Denk, H. Castrop, Generation of tubular superstructures by piling of renal stem/progenitor cells, Tissue Eng. Part C, Methods 1 (2008) 3–13.
[90] A. Roessger, L. Denk, W.W. Minuth, Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules, Biomaterials 30 (22) (2009) 3723–3732.
[91] J.M. Dufour, R.V. Rajotte, M. Zimmerman, A. Rezania, T. Kin, D.E. Dixon, G.S. Korbutt, Development of an ectopic site for islet transplantation, using biodegradable scaffolds, Tissue Eng. 9–10 (2005) 1323–1331.
[92] J.T. Borenstein, E.J. Weinberg, B.K. Orrick, C. Sundback, M.R. Kaazempur-Mofrad, J.P. Vacanti, Microfabrication of three-dimensional engineered scaffolds, Tissue Eng. 8 (2007) 1837–1844.
[93] J. Simon, R. de Petriconi, M. Meilinger, R.E. Hautmann, G. Bartsch, Optimized haemostasis in nephron-sparing surgery using small-intestine submucosa, BMC Urol 8 (2008) 8, doi:10.1186/1471-2490-8-8.
[94] E. Dawson, G. Mapili, K. Erickson, S. Taqvi, K. Roy, Biomaterials for stem cell differentiation, Adv. Drug Deliv. Rev. 2 (2008) 215–228.
[95] R. Stoop, Smart biomaterials for tissue engineering of cartilage, Injury (2008) S77–S87.
[96] P. Bawa, V. Pillay, Y.E. Choonara, L.C. Du Toit, Stimuli-responsive polymers and their applications in drug delivery, Biomed. Mater. (Bristol, England) 2 (2009) 22001, doi:10.1088/1748-6041/4/2/022001.
[97] Y. Bae, K. Kataoka, Intelligent polymeric micelles from functional poly(ethylene glycol)-poly(amino acid) block copolymers, Adv. Drug Deliv. Rev. 10 (2009) 768–784.
[98] M. Caldorera-Moore, N.A. Peppas, Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems, Adv. Drug Deliv. Rev. 15 (2009) 1391–1401.
[99] M.D. Krebs, O. Jeon, E. Alsberg, Localized and sustained delivery of silencing RNA from macroscopic biopolymer hydrogels, J. Am. Chem. Soc. 26 (2009) 9204–9206.
[100] Z. Zhang, Q. Zheng, J. Han, G. Gao, J. Liu, T. Gong, Z. Gu, Y. Huang, X. Sun, Q. He, The targeting of 14-succinate triptolide-lysozyme conjugate to proximal renal tubular epithelial cells, Biomaterials 7 (2009) 1372–1381.