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Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules

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ABSTRACT

The cell biological mechanism controlling the regeneration of renal tubules in renal failure after application of stem/progenitor cells is subject of actual research. Unsolved issues are the integration of stem/progenitor cells in a diseased organ environment, the differentiation into epithelial tissue and the formation of tubules in a spatial environment. Following this therapeutic strategy new biomaterials have to be found promoting spatial development of tubules. To obtain new information about the growth of tubules renal stem/progenitor cells from neonatal rabbit kidney were isolated and mounted in a tissue carrier between a selection of commercially available polyester fleeces. This procedure replaces coating by extracellular matrix proteins and creates an artificial interstitium supporting development of tubules. Perfusion culture was performed with chemically defined IMDM containing aldosterone as tubulogenic factor. Polyester fleeces were investigated by scanning electron microscopy. The spatial development of tubules was registered on whole-mount specimens and on cryosections labeled with SBA and antibodies indicating tubule differentiation. It is found that some polyester fleeces promote the spatial development of tubules between the fibers, whereat each of them produces its individual growth pattern.

1. Introduction

The application of stem/progenitor cells for the renewal of renal parenchyma in acute and chronic renal failure is in the focus of actual biomedical research [1]. For this kind of therapy stem/progenitor cells are applied by infusion over the capillary system [2] or by accidental injection into diseased parenchyma [3]. In both cases stem/progenitor cells have to migrate into diseased areas of the organ, where tubular cells undergo necrosis, apoptosis and detachment including denudation of the tubular basal lamina. At this unfriendly site stem/progenitor cells have to terminate the process of degradation and turn it in a process of regeneration. One of the essential presuppositions is the recreation of an optimal micro-environment suppressing inflammation, promoting cell proliferation with subsequent differentiation into functional tubules for the restoration. However, the limiting problem is that the molecular mechanisms involved in tubule formation are not known for normal kidney development and have consequently to be elaborated for the process of regeneration in a diseased kidney [4].

Previous experiments have shown that the development of embryonic cells into renal tubules is an unexpected complex chain of cell biological mechanisms consisting of epithelial development in combination with the control of spatial tube formation [5,6]. The development starts with the adherence of single cells and proceeds with controlled proliferation, sprouting and synthesis of a basal lamina until a polarized epithelium arises. In parallel the epithelium is forming a tube with a defined length and diameter comprising a straight or convoluted course. At present it is unclear, which factors trigger the elongation of a specific segment and the cellular communications involved in all of these processes.

Since the histoarchitecture of the kidney is complex, basic mechanisms of tubule formation can be best investigated under in vitro conditions. However, for this kind of experiments a powerful tissue culture system is needed [7–9]. Previous experiments demonstrated that culture of renal stem/progenitor cells at the interface of an artificial interstitium made by a I-7 polyester fleece results in the formation of numerous tubules [10,11]. The development of tubules is induced by aldosterone, depends on the applied hormone concentration and is antagonized by spironolactone or canrenoate [12].

Generation of tubules at the interface of an artificial polyester interstitium replaces the frequently used coating by extracellular matrix proteins [10]. Up to date it is unknown which biophysical features on the surface of the polyester fibers promote that tubules

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develop in a spatial manner. These pioneering experiments were performed with a I-7 polyester fleece measuring 0.59 mm in thickness. Other parameters such as water porosity and micron rating were not given by the provider. Thus, to obtain more information about promoting influences on the development of tubules a new set of Posi polyester fleeces was tested. In comparison to earlier used I-7 polyester fleece all of the Posi fleeces exhibit a decreased thickness between 0.13 mm and 0.18 mm. The Posi fleeces have a water porosity in a range from 700 L/m^2/S to 1480 L/m^2/S and the micron rating is between 15 and 40 (Table 1). For the first time it is shown that Posi polyester fleeces are promising candidates for the generation of renal tubules in future regenerative medicine.

2. Materials and methods

2.1. Explants containing renal stem/progenitor cells

For the present experiments one-day-old New Zealand rabbits were anesthetized with ether and killed by cervical dislocation as described earlier [13]. Both kidneys were removed and dissected from pole to pole in a ventral and dorsal part. By stripping off the capsula fibrosa with fine forceps, a thin embryonic tissue layer containing numerous epithelial stem/progenitor cells within collecting duct ampullae, mesenchymal cells and S-shaped bodies was harvested (Fig. 1a).

2.2. Set-up of a tissue carrier for perfusion culture

Isolated embryonic renal tissue has to be fixed in plane position to prevent damage during culture. This was obtained by placing renal stem/progenitor cells between two punched out layers of polyester fleece measuring 5 mm in diameter. This arrangement results in a sandwich-like configuration with the freshly isolated embryonic tissue in the center and layers of polyester fleece covering the upper and lower side (Fig. 1b). For mounting of renal stem/progenitor cells a base ring of a Minusheet® tissue carrier with 13 mm inner diameter was transferred to a perfusion culture container with horizontal flow characteristics (Minucells and Minutissue, Bad Abbach, Germany) (Fig. 1d). A polyester fleece measuring 13 mm in diameter was placed in the tissue carrier and the sandwich set-up configuration containing renal stem/progenitor cells was inserted. A further polyester fleece with 13 mm in diameter was then placed on top of the sandwich as a cover (Fig. 1c). After closing the lid of the perfusion culture container the layers of fleece keep the isolated tissue in flat position. The interface between the fleece layers produces an artificial interstitium promoting the spatial development of tubules during the entire culture period [10]. The spatial area for tubule formation was 5 mm in diameter and up to 250 μm in height.

2.3. Biomaterials

Renal stem/progenitor cells were kept at the interface of an artificial interstitium made of several kinds of polyester fleeces. Earlier used polyester fleece I-7 (Walraf, Grevenbroich, Germany) was tested in comparison with polyester fleeces Posi-4, Posi-5, Posi-6 and Posi-7 (Positech, Hallwil, Suisse) (Table 1).

2.4. Perfusion culture

Perfusion culture was performed as described earlier [11,14] (Fig. 1e). During a culture period of 13 days always fresh medium was transported with 1.25 ml/h by an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany). To maintain a constant temperature of 37 °C the perfusion culture container was placed on a thermoplate (Medax, Kiel, Germany) and covered with a transparent lid. For the generation of renal tubules chemically defined IMDM (Iscove's Modified Dulbecco's Medium including Phenolred, GIBCO/Invitrogen, Karlsruhe, Germany) was used. In order to sustain a constant pH of 7.4 under atmospheric air containing 0.3% CO_2 up to 50 mmol/l HEPES (GIBCO) was added to the medium. To induce tubulogenic development aldosterone (1×10^{-7} M, Fluka, Taufkirchen, Germany) was added to the culture medium. To impede infections an antibiotic-antimycotic cocktail (1%, GIBCO) was present in all culture media.

Table 1
Parameters of polyester fleeces used for the generation of renal tubules at the interface of an artificial interstitium. Except I-7 fleece data are given by the provider.

Manufacturer	Walraf	Positech			
Identification	**I-7**	**Posi-4**	**Posi-5**	**Posi-6**	**Posi-7**
Thickness [mm]	0.59	0.13	0.17	0.18	0.18
Water porosity [L/m^2/S]	–	1480	1200	1100	700
Micron rating [μm]	–	40	30	25	15

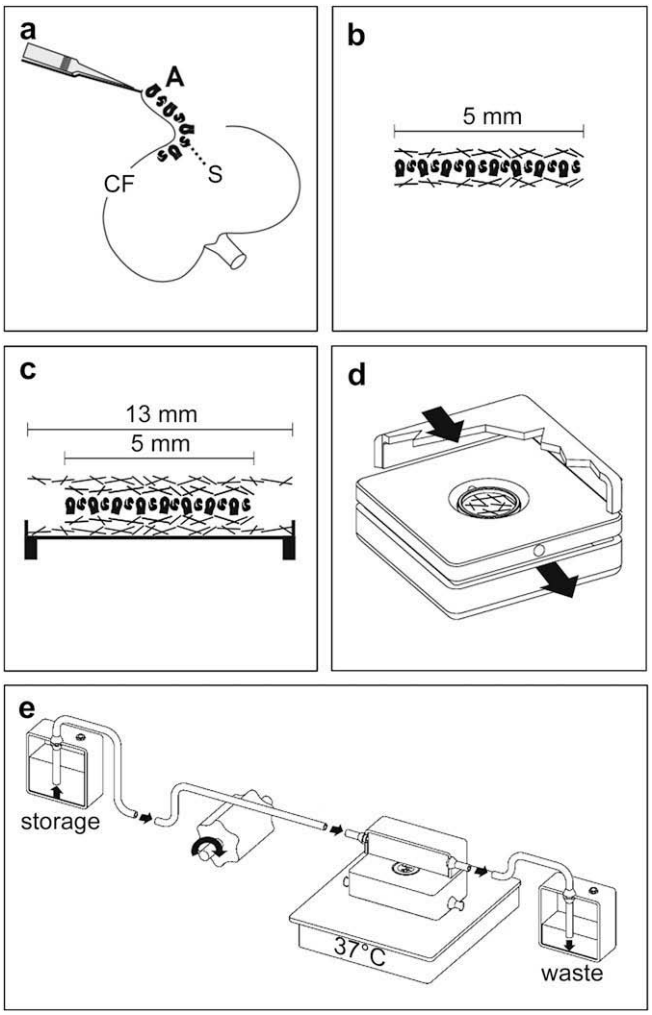

Fig. 1. Schematic illustration of isolating renal stem/progenitor cells and generation of tubules at the interphase of an artificial interstitium. Renal stem/progenitor cells are isolated by stripping off the capsula fibrosa from neonatal rabbit kidney (a). Isolated embryonic tissue is placed between two layers of polyester fleece like a sandwich (b). For stabilization the sandwich set-up is covered at the upper and lower side by fleeces mounted in a tissue carrier (c). Generation of tubules is performed in a perfusion culture container (d). Perfusion culture is performed with always fresh medium for 13 days at a rate of 1.25 ml/h with a peristaltic pump. To maintain a constant temperature of 37 °C the culture container is placed on a thermoplate and covered with a transparent lid (e).

2.5. Histochemical labeling

Following perfusion culture the sandwich set-ups containing renal tissue and two layers of polyester fleece (5 mm diameter) were embedded in 1% Agarose (Serva, Heidelberg, Germany), surrounded by TissueTek (O.C.T.™COMPOUND, Sakura Finetek, Zoeterwoude, Netherlands) and frozen at −80 °C. The fleeces measuring 13 mm in diameter were fixed in 70% ethanol to investigate them as whole-mount specimens. Both whole-mount specimens and 20 μm thick cryosections were first fixed in ice cold ethanol, washed several times with phosphate buffered saline (PBS) and incubated for 30 min with blocking solution (PBS, pH 7.5, 10% horse serum, GIBCO, 1% bovine serum albumin, Serva). For lectin-labeling the samples were exposed to fluorescein-isothiocyanate (FITC)-conjugated soybean agglutinin diluted 1:1000 (SBA, Vector, Burlingame, USA) in blocking solution for 45 min. Antibodies recognizing laminin γ1 (kindly provided by Dr. L. Sorokin, Lund, Sweden), TROMA-I (Developmental Studies Hybridoma Bank, DSHB, Iowa City, USA) and Na/K-ATPase alpha 5 (DSHB) were used undiluted, cingulin (Progen Biotechnik, Heidelberg, Germany) was applied 1:10 in blocking solution for 1 h. After washing with 1% BSA in PBS the specimens were incubated for 45 min with

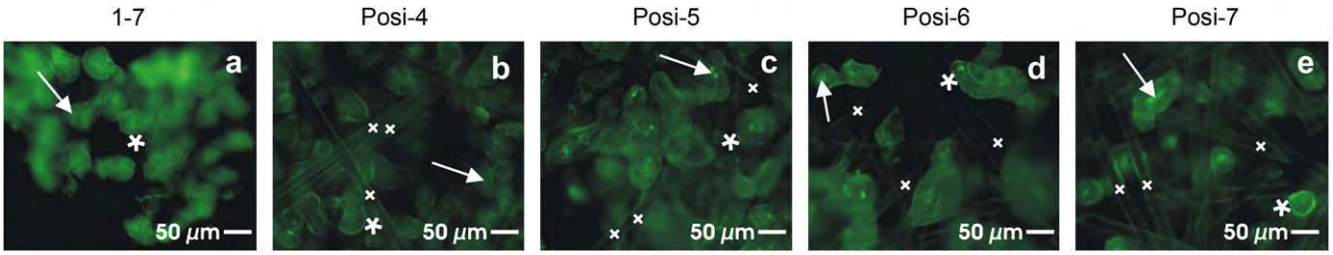

Fig. 2. Fluorescence microscopy on whole-mount specimens labeled with SBA. Tubules were generated within 13 days at the interface of an artificial interstitium made of I-7 (a), Posi-4 (b), Posi-5 (c), Posi-6 (d) and Posi-7 (e) polyester fleeces. SBA-label shows that polyester fibers (cross) are lacking between generated tubules using a I-7 fleece (a). In contrast, specimens generated on Posi-4 (b), Posi-5 (c), Posi-6 (d) and Posi-7 (e) fleeces show numerous fibers between generated tubules. These tubules exhibit a lumen (arrow) and a basal lamina (asterisk).

donkey-anti-mouse-IgG-fluorescein-isothiocyanate (FITC), goat-anti-rat-IgG-rhodamine or donkey-anti-guinea-pig-IgG-fluorescein-isothiocyanate (Jackson Immunoresearch Laboratories, West Grove, USA) diluted 1:50 in PBS containing 1% BSA. Following several washes with PBS the sections were embedded with Slow Fade Light Antifade Kit (Molecular Probes, Eugene, USA) 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 Corel DRAW 11 (Corel Corporation, Otawa, Canada).

2.6. Scanning electron microscopy

For scanning electron microscopy (SEM) the polyester fleeces were sputter-coated with gold (Polaron E 5100, Watford, GB). Then the specimens were examined in a scanning electron microscope DSM 940 A (Zeiss, Oberkochen, Germany) as described earlier [15]. Images of the screen were taken by a Pentax SLR Digital and thereafter processed with Adobe Photoshop 7.0 (Adobe, California, USA) and Corel DRAW 11 (Corel Corporation, Otawa, Canada).

2.7. Amount of cultured constructs

A total of 36 embryonic tissues were isolated and maintained in culture for the present study. All of the experiments were performed at least in triplicates. The data provided in the text are the mean of at least three independent experiments. All experiments are in accordance with the animal ethics committee, University of Regensburg, Regensburg, Germany.

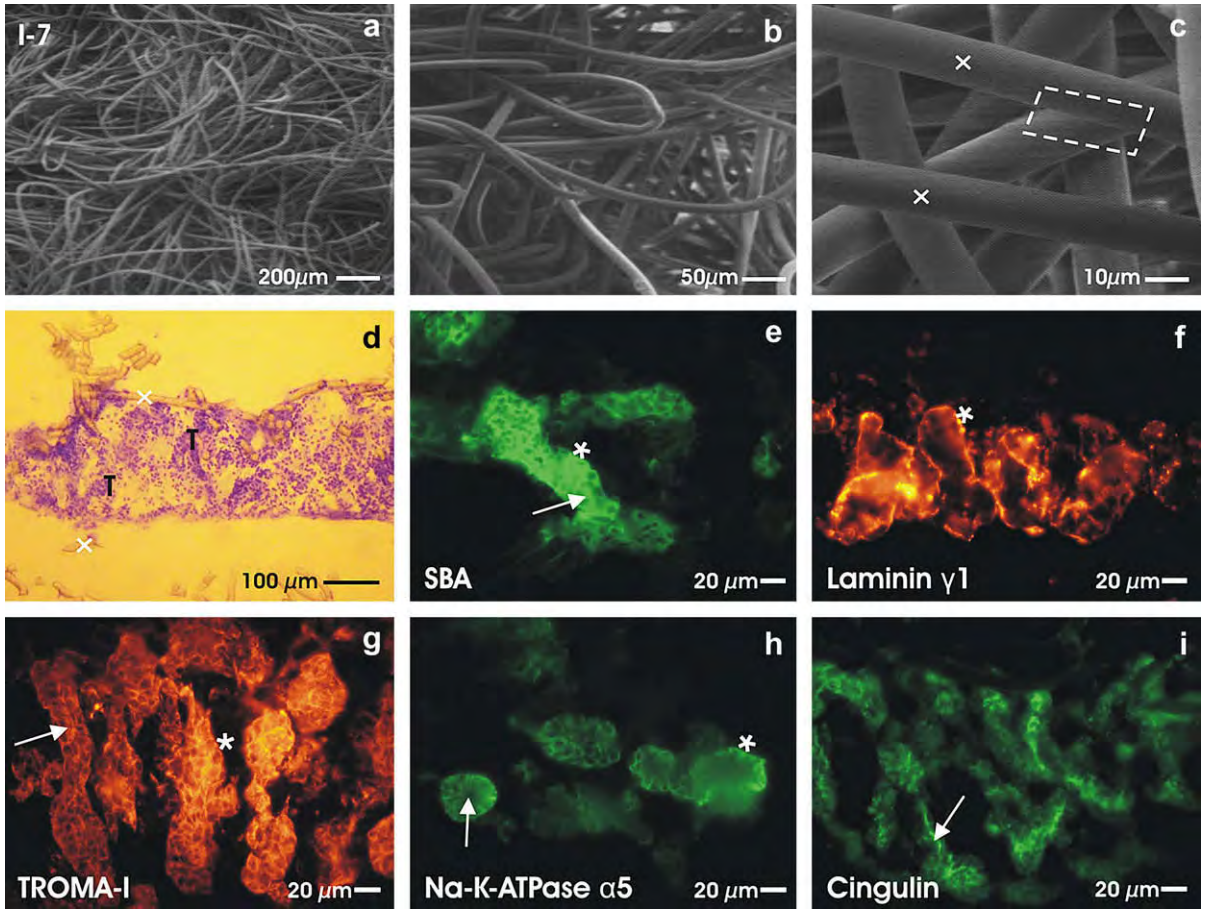

Fig. 3. SEM and histochemistry at the interphase of an I-7 artificial interstitium. The fibers of the polyester fleece are detected in a longitudinal, transversal and oblique course (a). They exhibit a homogeneous composition and a smooth surface without recognizable protrusions or roughness (b). Connections (rectangle) between single fibers are not detected (c). The diameter of fibers is 10 µm in average (a–c). Toluidine blue stained cryosections show numerous tubules (T) covered by fleece layers out of polyester fibers (cross) at the lower und upper side (d). Fibers between the tubules do not occur. Histochemical label for SBA (e), laminin γ1 (f), TROMA-I (g), Na/K-ATPase alpha 5 (h) and cingulin (i). Generated tubules exhibit a lumen (arrow) and a basal lamina (asterisk).

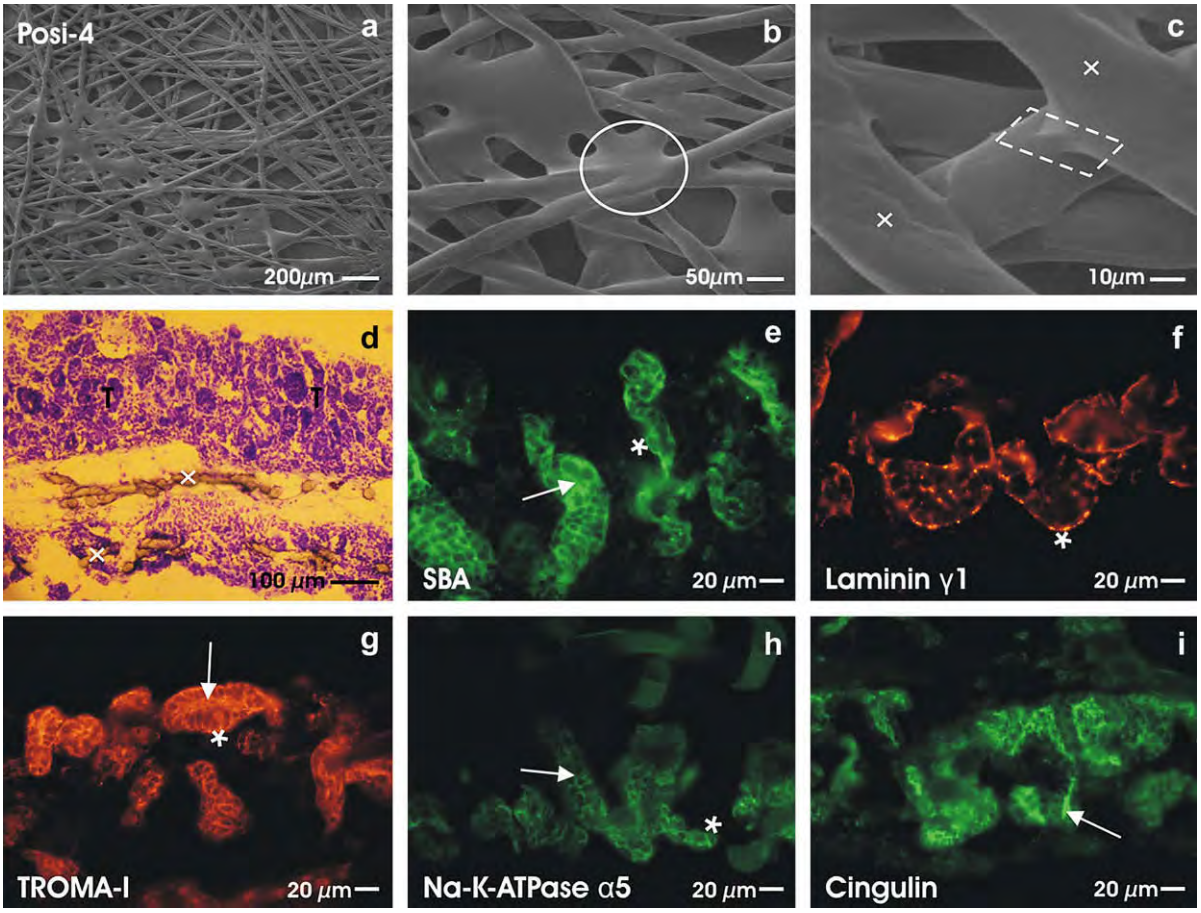

Fig. 4. SEM and histochemistry at the interphase of a Posi-4 artificial interstitium. The fibers (cross) of the polyester fleece are detected in a longitudinal, transversal and oblique course (a). They exhibit a homogeneous composition and a smooth surface without recognizable protrusions or roughness. Crossing points (circle) (b) and connections (rectangle) (c) between single fibers are observed. The diameter of fibers is 25 µm in average (a–c). Toluidine blue stained cryosections exhibit numerous tubules (T) at the fleece (d). Numerous fibers occur between the tubules. Histochemical label for SBA (e), laminin γ1 (f), TROMA-I (g), Na/K-ATPase alpha 5 (h) and cingulin (i). Generated tubules exhibit a lumen (arrow) and a basal lamina (asterisk).

3. Results

3.1. Generation of tubules

First, whole-mount label with SBA was performed to get information about the growth pattern of tubules generated in combination with polyester fleece I-7 (Fig. 2a), Posi-4 (Fig. 2b), Posi-5 (Fig. 2c), Posi-6 (Fig. 2d) and Posi-7 (Fig. 2e). Fluorescence microscopy demonstrates that in all specimens numerous labeled tubules are detected. All of them exhibit a basal lamina, lining epithelial cells and a visible lumen. However, the pattern of growth differs between I-7 fleece (Fig. 3) and Posi fleeces (Figs. 4–7). Using I-7 fleece fibers between the tubules cannot be observed (Fig. 3d), while samples generated with Posi-4 (Fig. 4d), Posi-5 (Fig. 5d), Posi-6 (Fig. 6d) and Posi-7 (Fig. 7d) fleeces demonstrate numerous fibers between generated tubules. To get more information about the different growth pattern between I-7 and Posi fleeces scanning electron microscopy of fleece surface (Figs. 3–7 a–c) and histochemistry on cryosections (Figs. 3–7 e–i) of generated tubules were performed.

3.2. Pattern with I-7 polyester fleece

Scanning electron microscopy in low (Fig. 3a), middle (Fig. 3b) and high (Fig. 3c) magnification of the polyester fleece I-7 demonstrates numerous fibers in a three-dimensional extension. The fibers of the polyester fleece are detected in a longitudinal, transversal and oblique course. They are of homogeneous composition and show a smooth surface without recognizable protrusions or roughness. Their average diameter is 10 µm. When fibers are crossing the sites of contact they do not show any remarkable feature. Toluidine blue labeled frozen sections of generated tissue demonstrate that numerous tubules are present at the interface of the artificial interstitium made with polyester fleece I-7 (Fig. 3d). The tubules are covered at the lower and at the upper side by polyester fibers of the fleece. It is obvious that the majority of tubules do not get in touch with polyester fibers. In few cases the basal lamina of the tubules is in contact with a polyester fiber. A close contact between the fibers and the epithelium is not observed. Histochemical label with SBA demonstrates the occurrence of numerous tubules (Fig. 3e). Immunohistochemical label for laminin γ1 reveals that the generated tubules exhibit a basal lamina (Fig. 3f). Positive label for TROMA-I (Fig. 3g) shows that generated tubules contain a cytoskeleton typically found in the collecting duct tubule within the kidney. Label for Na/K-ATPase alpha 5 (Fig. 3h) at the basolateral side of cells elucidates a typical feature of epithelial transport, while label for plaque protein cingulin (Fig. 3i) illustrates the formation of a tight junction between the apical and lateral plasma membrane.

3.3. Pattern with Posi-4 polyester fleece

Scanning electron micrographs of the polyester fleece Posi-4 in low (Fig. 4a), middle (Fig. 4b) and high (Fig. 4c) magnification show

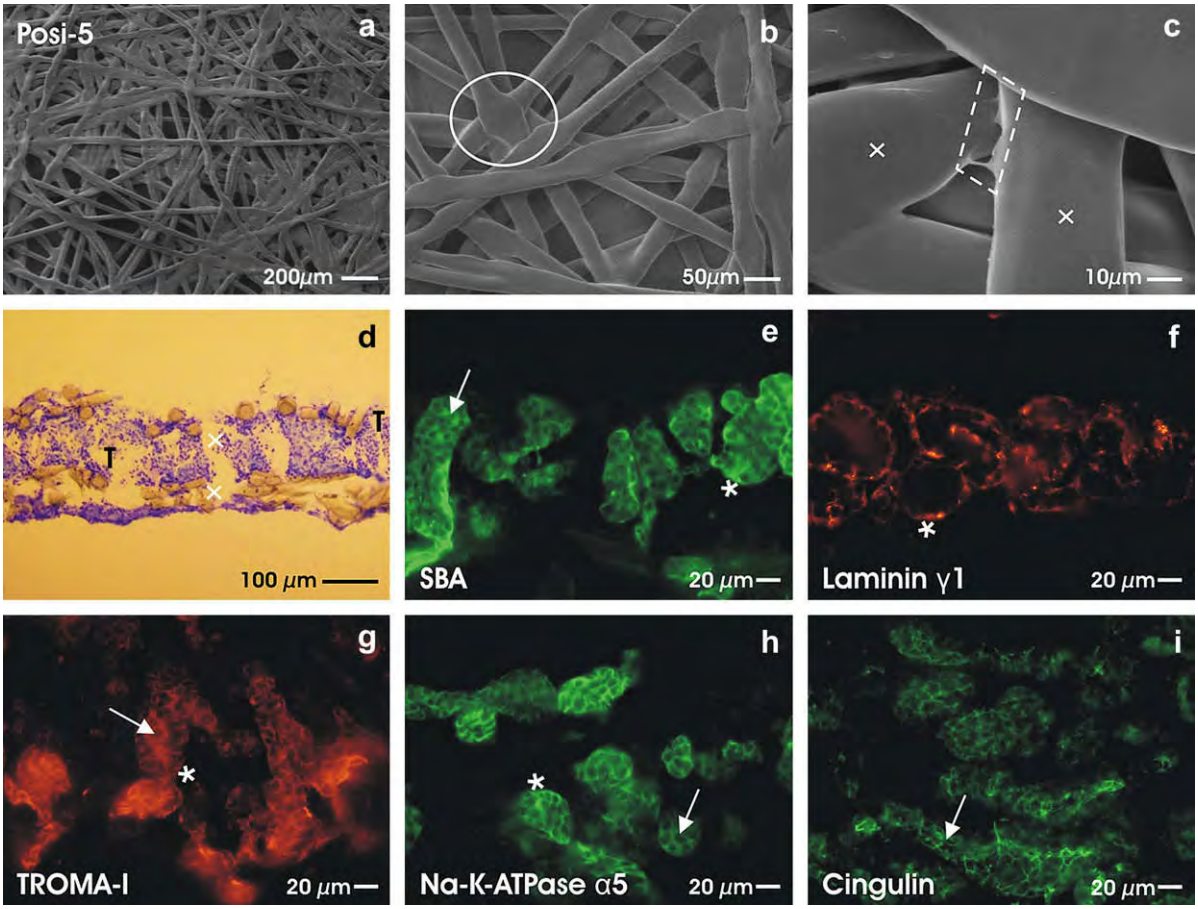

Fig. 5. SEM and histochemistry at the interphase of a Posi-5 artificial interstitium. The fibers of the polyester fleece are detected in a longitudinal, transversal and oblique course (a). They exhibit a homogeneous composition and a smooth surface without recognizable protrusions or roughness. Crossing points (circle) (b) and connections (rectangle) (c) between single fibers are observed. The diameter of fibers is 29 μm in average (a–c). Toluidine blue stained cryosections exhibit numerous tubules at the fleece. Numerous fibers occur between the tubules (d). Histochemical label for SBA (e), laminin γ1 (f), TROMA-I (g), Na/K-ATPase alpha 5 (h) and cingulin (i). Generated tubules exhibit a lumen (arrow) and a basal lamina (asterisk).

numerous fibers in three-dimensional extension. Constituent fibers are detected in longitudinal, transversal and diagonal course. The fibers have a meshed, smooth and unruffled surface. The average diameter of polyester fibers is 25 μm. The form of diameter is inconstant and varies from perfectly round to more or less oval. Most striking are points, where fibers are crossing each other. At these spots the fibers change their shape from round to oval and appear welded to each other (Fig. 4a). Higher magnification depicts that the space between two crossing fibers is filled by amorphous material (Fig. 4c). Cryosections of generated tissue demonstrate that an increased number of generated tubules is present using Posi-4 polyester fleece (Fig. 4d). In contrast to polyester fleece I-7 (Fig. 3d) the fibers of Posi-4 do not cover the generated tissue at the upper and lower side but are found to be widely distributed between the tubules (Fig. 4d). In many cases the basal lamina of the tubules is in contact with the fibers, but an integration of the fibers into the epithelium is not observed. Histochemical label for SBA shows the presence of numerous generated tubules (Fig. 4e). Immunohistochemical label for laminin γ1 demonstrates that generated tubules contain laminin in the basal lamina (Fig. 4f). Positive label for TROMA-I shows that generated tubules express a typical cytoskeletal protein (Fig. 4g). Label for Na/K-ATPase alpha 5 at the basolateral plasma membrane elucidates a typical feature of epithelial transport (Fig. 4h). The presence of cingulin exhibits the development of a tight junctional complex (Fig. 4i).

3.4. Pattern with Posi-5 polyester fleece

Scanning electron microscopy in low (Fig. 5a), middle (Fig. 5b) and high (Fig. 5c) magnification of the polyester fleece Posi-5 reveals numerous fibers extending in a longitudinal, transversal and oblique course. All of the fibers appear to be homogeneous in composition. They exhibit a smooth surface without recognizable protrusions or roughness. Their diameter ranges from 29 μm to 34 μm. The shape of fibers appears occasionally oval. Most conspicuous are the crossing points of the fibers. In these areas the fibers are flattened as compared to fibers showing a straight course (Fig. 5a). Higher magnification demonstrates that fibers in points of contact are flat and appear to be welded to each other (Fig. 5c). Cryosections of cultures display that an increased number of tubules is generated using Posi-5 polyester fleece (Fig. 5d). In most of the cases the basal lamina of tubules is in contact with the fibers. However, an integration of the fibers into the epithelium is not observed in the series with Posi-5. SAB-label shows an intensive reaction on generated tubules (Fig. 5e) Immunohistochemical label for laminin γ1 demonstrates the synthesis of a basal lamina (Fig. 5f). Positive label for TROMA-I shows the presence of a typical feature of the cytoskeleton (Fig. 5g), while label for Na/K-ATPase alpha 5 indicates epithelial transport (Fig. 5h). Label for cingulin reveals the presence of a tight junction (Fig. 5i).

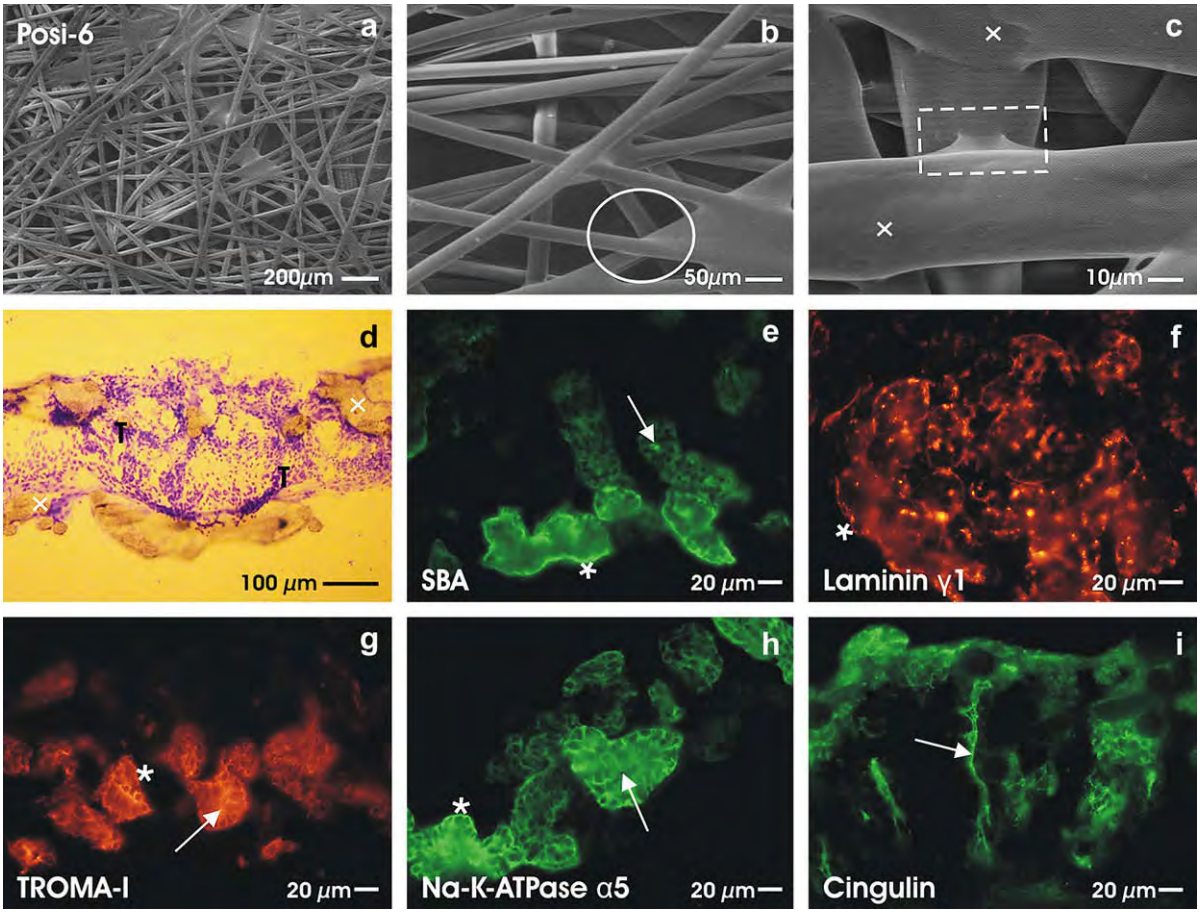

Fig. 6. SEM and histochemistry at the interphase of a Posi-6 artificial interstitium. The fibers of the polyester fleece are detected in a longitudinal, transversal and oblique course (a). They exhibit a homogeneous composition and a smooth surface without recognizable protrusions or roughness. Crossing points (circle) (b) and connections (rectangle) (c) between single fibers are observed. The diameter of fibers is 24 µm in average (a–c). Toluidine blue stained cryosections exhibit numerous tubules at the fleece. Numerous fibers occur between the tubules (d). Histochemical label for SBA (e), laminin γ1 (f), TROMA-I (g), Na/K-ATPase alpha 5 (h) and cingulin (i). Generated tubules exhibit a lumen (arrow) and a basal lamina (asterisk).

3.5. Pattern with Posi-6 polyester fleece

Low (Fig. 6a), middle (Fig. 6b) and high (Fig. 6c) magnification in the scanning electron microscope exhibits that polyester fleece Posi-6 is composed out of numerous fibers spreading in flat extension. Similar to Posi-4 (Fig. 4a) and Posi-5 (Fig. 5a) the fibers show a longitudinal, transversal and oblique course. All of the fibers appear to be homogeneously composed and reveal a smooth surface without recognizable protrusions or roughness. Their average diameter is 24 µm. The form of diameter is constant and appears almost round. Most striking are the points, where fibers are crossing. Here several fibers are attached to each other. In contrast to Posi-4 (Fig. 4b) and Posi-5 (Fig. 5b) in Posi-6 series the shape of fiber at the contact site remains round (Fig. 6b). In contrast, the space between crossing fibers is filled with amorphous material (Fig. 6b). Higher magnification demonstrates that fibers at contact sites stay round and appear to be connected by amorphous material (Fig. 6c). Cryosections reveal that in contrast to Posi-4 and Posi-5 a decreased number of generated tubules is found in series with Posi-6 polyester fleece (Fig. 6d). The generated tissue is not capped at the upper and lower side by fibers of Posi-6 fleece. Thus, fibers are located between the generated tubules (Fig. 6d). In the majority of cases the basal lamina of tubules is in contact with the fibers. An integration of the fibers into the epithelium is not observed. SBA-label shows an intensive reaction on developed tubules (Fig. 6e). Immunohistochemical label for laminin γ1 depicts that the generated tubules contain a basal lamina (Fig. 6f), while positive label for TROMA-I shows the synthesis of a typical cytoskeleton (Fig. 6g). Label for Na/K-ATPase alpha 5 indicates a typical feature of epithelial transport that is found at the basolateral plasma membrane (Fig. 6h). The presence of cingulin shows the formation of a tight junctional complex (Fig. 6i).

3.6. Pattern with Posi-7 polyester fleece

Scanning electron micrographs of the polyester fleece Posi-7 in low (Fig. 7a), middle (Fig. 7b) and high (Fig. 7c) magnification show numerous fibers in three-dimensional extension. Constituent fibers are detected in longitudinal, transversal and oblique course. The average diameter of polyester fibers is 29 µm. The form of diameter is inconstant and varies from perfectly round to oval. Most striking are points, where fibers are crossing each other. At these spots the fibers change their shape from round to oval and appear welded to each other (Fig. 7b). Higher magnification depicts that the space between two crossing fibers is filled by amorphous material (Fig. 7c). Cryosections of generated tissue with Posi-7 fleece (Fig. 7d) demonstrate that a number of generated tubules is present as it is the case with Posi-6 (Fig. 6d). In contrast to polyester fleece I-7 (Fig. 3d) the fibers of Posi-7 do not cover the generated tissue at the upper and lower side but are found to be widely distributed

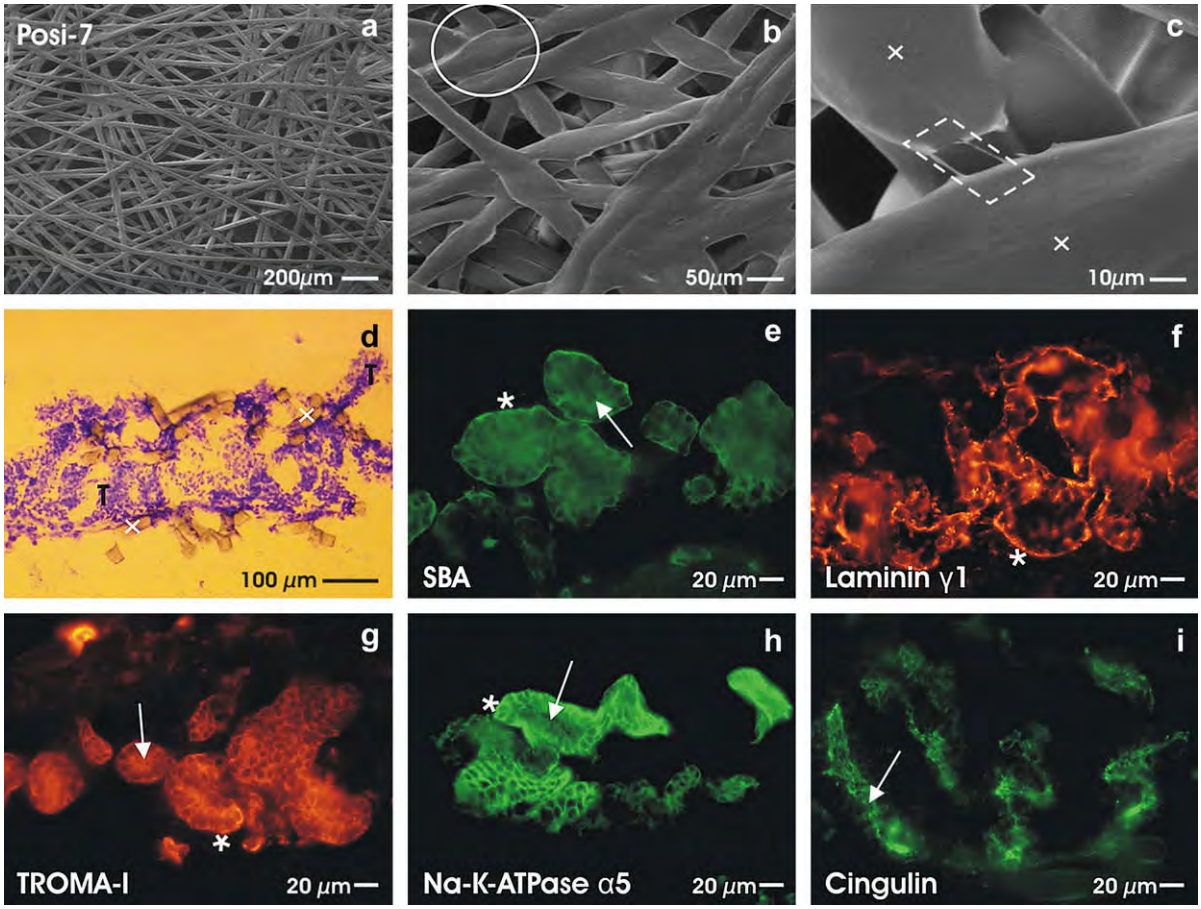

Fig. 7. SEM and histochemistry at the interphase of a Posi-7 artificial interstitium. The fibers of the polyester fleece are detected in a longitudinal, transversal and oblique course (a). They exhibit a homogeneous composition and a smooth surface without recognizable protrusions or roughness. Crossing points (circle) (b) and connections (rectangle) (c) between single fibers are observed. The diameter of fibers is 29 μm in average (a–c). Toluidine blue stained cryosections exhibit numerous tubules at the fleece. Numerous fibers occur between the tubules (d). Histochemical label for SBA (e), laminin γ1 (f), TROMA-I (g), Na/K-ATPase alpha 5 (h) and cingulin (i).

between the tubules (Fig. 7d). An integration of the fibers in the epithelium is not observed in series using a Posi-7 fleece. In few cases the basal lamina of tubules is in contact with the fibers. Histochemical label for SBA shows the presence of numerous generated tubules (Fig. 7e). Immunohistochemical label for laminin γ1 demonstrates that generated tubules have synthesized a basal lamina (Fig. 7f). Positive label for TROMA-I shows that generated tubules express a typical protein of the cytoskeleton (Fig. 7g). Label for Na/K-ATPase alpha 5 at the basolateral plasma membrane illuminates a typical feature of epithelial transport (Fig. 7h), while label for cingulin depicts the formation of a tight junctional complex (Fig. 7i).

4. Discussion

The challenging aim in future regenerative medicine is the treatment of acute and chronic renal failure by the help of stem/progenitor cells. Up to date unsolved issues are an optimal application of stem/progenitor cells and their integration into diseased parenchyma inducing here the regeneration process.

4.1. Application of stem/progenitor cells in a diseased kidney

The frequently performed infusion technique of stem/progenitor cells has the advantage that the cells are widely distributed over the capillary system (Fig. 8a) [2,16]. However, infused cells are found in healthy and diseased areas of the parenchyma so that a concentration at the site of necessary regeneration is not obtained. In addition, infused stem/progenitor cells have to pass the endothelial barrier until they reach the interstitial space to restore diseased parenchyma. The traditional intravenous route of administration for stem/progenitor cells may result consequently in an inefficient availability of donor cells, possibly contributing to suboptimal engraftment and high risk of graft versus host reaction [17].

An alternative is the microsurgical implantation of stem/progenitor cells in the renal parenchyma, where they undergo growth and differentiation at the site of damage (Fig. 8b) [18,19]. However, disadvantage of this method is given by the numerous foci of degeneration that cannot all be reached by an accidental spot implantation so that the number of implanted cells in a diseased organ remains inconstant and concentration is not obtained at sites of need.

A third contribution for repair in kidney injury is the subcapsular implantation of stem/progenitor cells (Fig. 8c) [20]. The overall hypothesis is that the implanted cells activate regeneration at the site of earlier renal stem cell niche. This area is found in the embryonic cortex containing collecting duct ampullae (epithelial stem cells) and surrounding nephrogenic mesenchyme (nephrogenic mesenchymal stem cells) [21,22]. Initiated by the reciprocal interaction between both stem cell populations the formation of all nephrons is started but also terminated [23]. Thus, the cortex

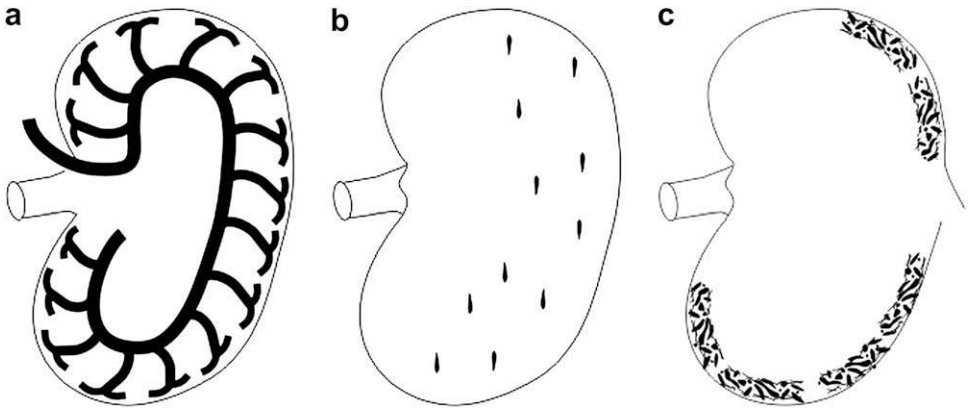

Fig. 8. Schematic illustration of possible methods to apply stem/progenitor cells by infusion (a), by implantation of cell suspensions in the parenchyma (b) or by subcapsular implantation of biomaterial with adherent stem/progenitor cells (c).

corticis is the area, where the last generation of nephrons was formed. It is also the border, where the organ has reached its final size. Up to date cells for repair are injected in the subcapsular space either as suspension [24] or after coating by extracellular matrix proteins [25].

4.2. Binding stem/progenitor cells to a biomaterial before implantation

The subcapsular implantation of stem/progenitor cells to the site of earlier nephron formation appears in our sight as most challenging technique. In so far we follow an improved strategy. The idea is not to administer a suspension but to adhere stem/progenitor cells to a biomaterial, which is implanted between the capsule and the kidney cortex. Thus the material has to be biocompatible with both adult renal parenchyma and stem/progenitor cells. The specific site of implantation requires further a minimal thickness of the biomaterial with adherent stem/progenitor cells so that it can be shoved in the narrow cleft between the renal capsule and the superficial nephrons. Most important, the selected support material has to exhibit certain stiffness, has to stabilize the adherent stem/progenitor cells and must promote the process of regeneration including vascularization after implantation in a diseased environment.

However, independently from the future use of embryonic stem cells, organ specific stem cells or other types of progenitor cells a suitable support material for the adherence of stem/progenitor cells has to be found and critically tested by sophisticated culture technique before any surgical implantation is performed. Previous and present culture experiments demonstrate that numerous renal tubules can be generated between two layers of I-7 polyester fleece (Fig. 2a) [26,11,27]. The interface between the fleece layers produces an artificial interstitium promoting the spatial development of tubules. The I-7 polyester fleece exhibits excellent biocompatibility. However, hindering is its thickness. For that reason Posi fleeces were evaluated, if they promote the generation of tubules. The Posi fleeces consist of pure polyester and exhibit a thickness between 0.13 and 0.18 mm as compared to 0.59 mm of earlier used I-7 fleece (Table 1).

4.3. Varying growth pattern in polyester fleeces

Perfusion culture experiments demonstrate that the application of Posi-4 (Fig. 2b), Posi-5 (Fig. 2c), Posi-6 (Fig. 2d) and Posi-7 (Fig. 2e) fleeces results in the generation of numerous tubules. As revealed by whole-mount label all of the Posi fleeces exhibit excellent features for development of tubules. Toxic effects cannot be observed. Immunohistochemistry of specimens applying Posi-4 (Fig. 4e–i), Posi-5 (Fig. 5e–i), Posi-6 (Fig. 6e–i) and Posi-7 (Fig. 7e–i) fleeces further demonstrate that the generated tubules exhibit the same pattern of cell biological differentiation profile as demonstrated with I-7 fleece (Fig. 3e–i).

In contrast, the spatial pattern of tubule growth in Posi fleeces is different (Fig. 9b–e) in comparison to previously used I-7 polyester fleece (Fig. 9a). Whole-mount label of specimens generated with I-7 fleece (Fig. 2a) do not exhibit any polyester fibers between growing tubules as it is the case with Posi fleeces (Fig. 2b–e). Cryosections support this finding, since in series with I-7 fleece growth of tubules is exclusively observed at the artificial interstitium produced between the layers of fleeces (Fig. 3d). In this case development of tubules between the fibers of the fleece is not observed. However, numerous fibers between tubules are detected, when Posi fleeces were applied (Figs. 4d,5d,6d,7d). Cryosections of Posi-4 (Fig. 4d–i), Posi-5 (Fig. 5d–i), Posi-6 (Fig. 6d–i) and Posi-7

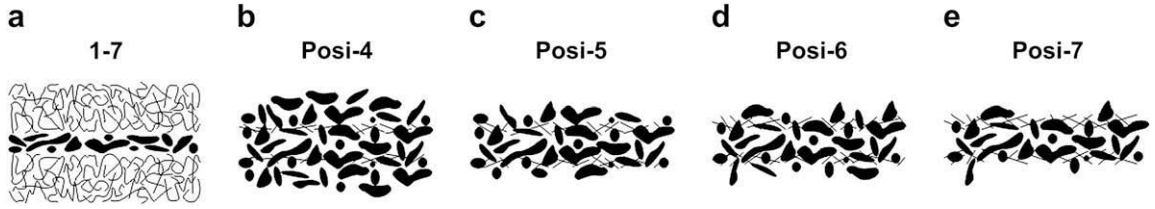

Fig. 9. Schematic illustration of growth pattern of tubules generated on an artificial interstitium made of I-7 (a), Posi-4 (b), Posi-5 (c), Posi-6 (d) and Posi-7 (e) polyester fleeces. With I-7 fleece growth of tubules is only observed at the artificial interstitium and not between the fibers of the fleece (Fig. 9a). With Posi-4 fleece growth of tubules occurs at the interface between the fleece layers, between the fibers and in wider neighborhood to the fibers (Fig. 9b). With Posi-5 (Fig. 9c), Posi-6 (Fig. 9d) and Posi-7 (Fig. 9e) growth of tubules occurs at the artificial interstitium and between the fibers.

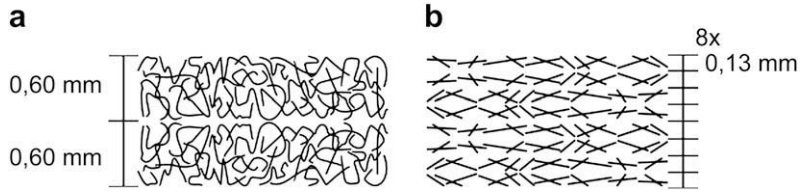

Fig. 10. Schematic illustration of pilled I-7 fleece (a) and pilled Posi-4 fleece (b).

(Fig. 7d–i) specimens demonstrate that growth of tubules is not only promoted at the interface between the fleeces' layers but also occurs between the fibers of each fleece. One reason could be the different diameter of the fleece fibers, which is at least 2.5× larger in Posi fleeces than in I-7. This fact could result in a better stability of the Posi fleece fibers which could promote by an unknown mechanism the development of tubules amongst the fibers.

Another factor for the growth of tubules inbetween the fibers could be their different crosslinking, which is performed mechanically in I-7 and in Posi fleeces it is done by heat. Calefaction could have influenced the surface of the fibers in a manner which could promote the growth of tubules among the fibers. Elaboration of the surface and molecular chemistry of the used fleeces were not given by the providers. Comparing scanning electron micrographs of I-7 and Posi polyester fleeces shows a difference concerning pores. In I-7 the pores do not got through the fleece from the upper to the reverse side. In Posi polyester fleeces lancing of pores can be observed. Thereby tubules could grow amongst the fibers without any barrier.

Various thickness, unequal diameter and crosslinking of the fibers and different pore lancing result in a diverse surface of the Posi fleeces compared to I-7 fleece. These could be given as the only reasons for the growth of tubules between the fibers. The fleece material cannot be the cause for the difference in spatial growth because both I-7 and the Posis are out of 100% polyester.

Furtheron, the density of tubule growth in Posi fleeces is not identical. In series with Posi-4 (Fig. 4d) fleeces development of tubules occurs not only in the space between the fibers, but is also extensively found in a sprouting manner in the wider neighborhood. This contrast experiments with Posi-5 (Fig. 5d), Posi-6 (Fig. 6d) and Posi-7 (Fig. 7d) fleeces. Here the growth of tubules is restricted to the close vicinity of polyester fibers. An explanation for the different pattern of growth cannot be given although the fleeces consist of the same material.

Although the development of tubules in the wider neighborhood is only found in series with Posi-4 fleece data cannot be correlated with biophysical properties such as thickness, water porosity and micron rating (Table 1). Besides thickness, water porosity and micron rating Posi-4, Posi-5, Posi-6 and Posi-7 fleeces differ in diameter of the fibers and in crossing points of fibers.

Finally, the unique growth pattern of renal tubules in Posi-4 (Fig. 4), Posi-5 (Fig. 5), Posi-6 (Fig. 6) and Posi-7 (Fig. 7) fleeces is the base for further cell biological experiments related to the formation of tubules. The demonstrated results exhibit a realistic chance for a future surgical application in regenerative medicine. For example, the minimal thickness of 0.13 mm in a Posi-4 in contrast to I-7 fleece (Fig. 10) makes piling of stem/progenitor cells possible and increases efficiently thereby the amount of implanted mass of cells. Furtheron, the presented experiments show the feasibility to regenerate renal tubules in combination with Posi-4 (Fig. 4), Posi-5 (Fig. 5), Posi-6 (Fig. 6) and Posi-7 (Fig. 7) fleeces. This again is an important presupposition to investigate further mechanisms steering development and vascularization of regenerating tubules under controlled culture conditions.

5. Conclusions

Various types of polyester fleece were tested due to the regeneration of renal tubules. In comparison to earlier tested materials Posi fleeces exhibit a decreased thickness in a range of 0.13 and 0.18 mm and show excellent features for the spatial development of tubules. The spatial growth of tubules does not only occur at the surface but also in the space between the fleece fibers. These specific characteristics demonstrate that Posi fleeces are appropriate novel candidates for the regeneration of parenchyma in diseased organs.

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