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Renal Tubular- and Vascular Basement Membranes and their Mimicry in Engineering Vascularized Kidney Tubules

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The high prevalence of chronic kidney disease leads to an increased need for renal replacement therapies. While there are simply not enough donor organs available for transplantation, there is a need to seek other therapeutic avenues as current dialysis modalities are insufficient. The field of regenerative medicine and whole organ engineering is emerging, and researchers are looking for innovative ways to create (part of) a functional new organ. To biofabricate a kidney or its functional units, it is necessary to understand and learn from physiology to be able to mimic the specific tissue properties. Herein is provided an overview of the knowledge on tubular and vascular basement membranes' biochemical components and biophysical properties, and the major differences between the two basement membranes are highlighted. Furthermore, an overview of current trends in membrane technology for developing renal replacement therapies and to stimulate kidney regeneration is provided.

1. Introduction

Worldwide more than 10% of the population is affected by chronic kidney disease (CKD).[1] This can progress until end-stage renal disease (ESRD) for which the preferred treatment is organ transplantation. Yearly, worldwide over 80 000 kidney transplants are being performed.[2] The high prevalence of ESRD leads to long waiting lists for donor organs. On average, a kidney patient has to wait 3–4 years before transplantation. In the meantime, kidney patients have to deal with renal replacement therapies (RRTs) such as dialysis, which are only partly capable of replacing kidney function and associated with severe side effects such as cardiovascular complications.[3,4] These side effects are, among others, a consequence of the insufficient clearance of uremic toxins, which are metabolic waste products that are in part coupled to plasma proteins such as albumin. Due to their physicochemical properties (large size), these protein-bound uremic toxins cannot be cleared via dialysis and lead to progression of CKD and cardiovascular diseases. As current dialysis therapies have not made significant improvements in the past decades, alternative innovative therapies are warranted. The emergent field of tissue engineering focuses on development of (parts of) bioartificial organs, e.g., by 3D bioprinting, which may yield a future solution for the shortage of kidney transplants. Although it might still take many years before a fully functional kidney can be fabricated, because of its complexity, important steps toward small functional parts of the organ are currently being made. To enable the creation of biologically active (parts of) organs, fundamental understanding of the organ and its physiological properties is essential.

The kidney plays a key role in maintaining body homeostasis by removing wastes from the blood, regulating blood pressure and electrolyte concentrations, by the production of hormones, and maintaining acid-base balance and extracellular fluid volume. These characteristics are fulfilled by many different cell types within the functional units of the kidney, termed nephrons. Nephrons are composed of five major segments, starting from the glomerulus (or Bowman's capsule), to the proximal kidney tubule, the loop of Henle, the distal kidney tubule, ending in the collecting duct. The removal of protein-bound uremic toxins depends on the activity of transport proteins present in renal tubular cells. This function cannot be replaced by current RRTs. Ongoing research, including from

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our group, aims to engineer the proximal kidney tubule, which would open a new era of implantable constructs that can replace renal function in vivo. The luminal surface of the tubular epithelial cells is covered with microvilli that highly increase their surface area, thereby facilitating the reabsorptive function. The proximal tubule contributes to body homeostasis via the reabsorption of 60%–70% of water and salts, and almost all of the nutrients in the ultrafiltrate.[5] Additionally, the proximal tubule is responsible for active solute secretion, from blood into the prourine, facilitated by transport proteins. Underneath the epithelial cells, a basement membrane (BM) is located, which provides structural support, but it is also involved in controlling growth factor signaling thereby supporting the tissue's function.[6] BMs are highly specialized extracellular matrices (ECMs) and act as scaffolds for the cells, as well as barriers. BMs are dynamic as they are continuously synthesized and degraded by cells, in a well-balanced process, and the composition of BMs can change over the course of renal development and disease.

To create a functional kidney tubule, proximal tubule cells have to be implemented in an (engineered) scaffold that serves as the BM for these cells. In the next chapters, we provide insight into the physiological conditions of the proximal tubular BM (TBM) and vascular BM (VBM), as well as their interplay, to create an overview on the important properties that should be included in a suitable scaffold.

2. The Role of the BM in Kidney Tubules

BMs are specialized sheet-like ECM structures that serve many functions including providing of mechanical stability and involvement in the regulation of essential cellular characteristics and growth factor signaling. BMs offer a structure on which both epithelial and endothelial cells can reside. The interaction between cells and BMs is of high importance for the development of tissues, their homeostasis, and also for the response to injury.[7] The macromolecular composition as well as the stiffness of the BM can affect the interactions between cells and the BM.[6,8,9] Defects of BM components have been associated with kidney diseases and/or adverse renal outcome.[7,10–12] Kidney tubule epithelial cells are attached to the TBM, which is an adhesive substrate that anchors the basal plasma membranes of cells attached.[13]

2.1. Biochemical and Biophysical Characteristics of the TBM

The BM consists of the lamina densa, an electron-dense zone, and the lamina rara or lucida, an electron-lucent zone.[14,15] **Figure 1** and **Table 1** show the main components of the BM that include collagen type IV, laminins, glycoproteins including nidogens and heparan sulfate proteoglycans (HSPGs) perlecan and agrin, and fibronectin.[6,7,13,16–18] Although these basic components are present in many tissues throughout the body, the exact composition of the ECM including the BM is very specific for every organ. Proteomic analysis revealed that the kidney ECM contains ≈220 proteins that are kidney specific.[19] The molecular heterogeneity of the renal BM is often linked to its segmental nature, and its components vary profoundly between

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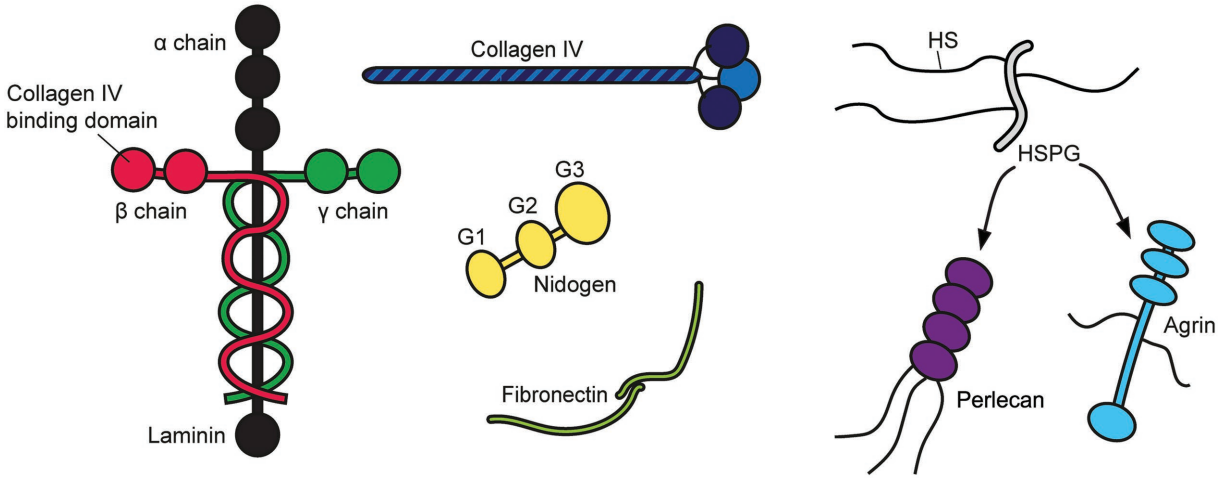

Figure 1. Schematic overview of the main components of the TBM and VBM: collagen type IV, laminin, nidogen, HSPGs perlecan and agrin, and fibronectin.

the nephron segments.[16] In this review article, we primarily focus on the proximal TBM.

Renal tubular epithelial cells are prone to lose their characteristics when cultured under standard in vitro conditions.[20] One of the reasons might be that while the ECM and BM promote many cell functions, including proliferation and differentiation via their mechanical and physical properties and stimuli, an ECM or BM-like structure is often absent in vitro. Therefore, the establishment of suitable BMs that can be used in vitro is of great importance to optimize the culture conditions for these cells and to improve physiological relevance.[21]

2.1.1. Biochemical Components

The molecular composition of the BM changes over time, especially during embryogenesis the molecular composition deviates from that of mature BM.[22] To form the tubules, mesenchymal cells have to convert to epithelial tubules, and the ECM changes into a BM. The components of the BM are mostly produced by the epithelial and endothelial cells that reside at the BM.

Table 1. Overview of the main components of the TBM and VBM.

Component	TBM	VBM	Functions	References
Collagen IV	$\alpha1$(IV)/$\alpha2$(IV) network	$\alpha1$(IV)/$\alpha2$(IV) network		[16,145]
Laminin	Laminin-1 ($\alpha1\beta1\gamma1$) Laminin-2 ($\alpha2\beta1\gamma1$) Laminin-10 ($\alpha5\beta1\gamma1$)	Laminin-2 ($\alpha2\beta1\gamma1$) – Larger vessels Laminin-8 ($\alpha4\beta1\gamma1$) Laminin-10 ($\alpha5\beta1\gamma1$)		[16,82,146–150]
Nidogen	Nidogen 1 Nidogen 2	Nidogen 1 Nidogen 2	Binds tightly laminin $\gamma1$ chain and collagen IV	[42,44]
Perlecan	Expressed	Expressed	Binds to nidogen, and collagen IV, involved in angiogenesis	[31,151]

Collagen Type IV: Collagen type IV is the most abundant component within TBMs comprising 50% or more of the total protein content.[13,23] Its molecular weight is ≈500 kDa. Typical for collagens is their triple helical structure that forms fibers that are cross-linked in a specific pattern within the BM, by which they form a flexible meshwork (**Figure 2**).[9] This meshwork contains pores with diameters that are slightly smaller than albumin molecules, and thereby function as size barrier. Collagen IV consists of three α chains that together form the triple helix. Within the collagen IV family there are three different protomers, which vary in distinct α chains that trimerize. The three protomers are $(\alpha1)_2\alpha2$, $\alpha3\alpha4\alpha5$, and $(\alpha5)_2\alpha6$. Within the ECM, the protomers self-polymerize to create a flexible network, which is crucial for the stability of the BM.[24]

Collagen IV is cross-linked via sulfilimine bonds (S=N) between methionine sulfur and the nitrogen of hydroxylysine.[25] The links are formed by the matrix enzyme peroxidasin. Bhave et al. recently showed that in a mouse model with reduced collagen IV sulfilimine cross-links, a reduction in renal TBM stiffness was observed.[8]

Laminins: Laminins are heterotrimers that consist of one α chain, one β chain, and one γ chain (Figure 1). Since there are various α, β, and γ chains, a total of 16 laminin isoforms have been identified. Laminins have a high-molecular weight and play a major role in BM assembly.[26] The self-assembly of laminin takes place at the cell surface, where it is anchored to the cell through interactions with receptors. Opposed to the BM of other organs, laminin-1 accumulates in the renal BM at all stages of development, from embryonic to mature renal tissue.[16,27] Madri et al. studied the localization of laminin in the murine renal BM, where laminin was located in the mesangial areas of the glomerulus as well as in typical BM-like patterns on both the glomerular BM (GBM) and TBM.[28] Furthermore, laminin was mainly located at the same sites as the collagens.[28] Laminin is located on the endothelial side of the BM, where it is suggested to be synthesized by endothelial cells that subsequently use it for adhesion to the underlying BM, or it might play a role as a glycoprotein that contains acidic residues and sialic acid. Laminin-1 ($\alpha1\beta1\gamma1$), laminin-2 ($\alpha2\beta1\gamma1$), and

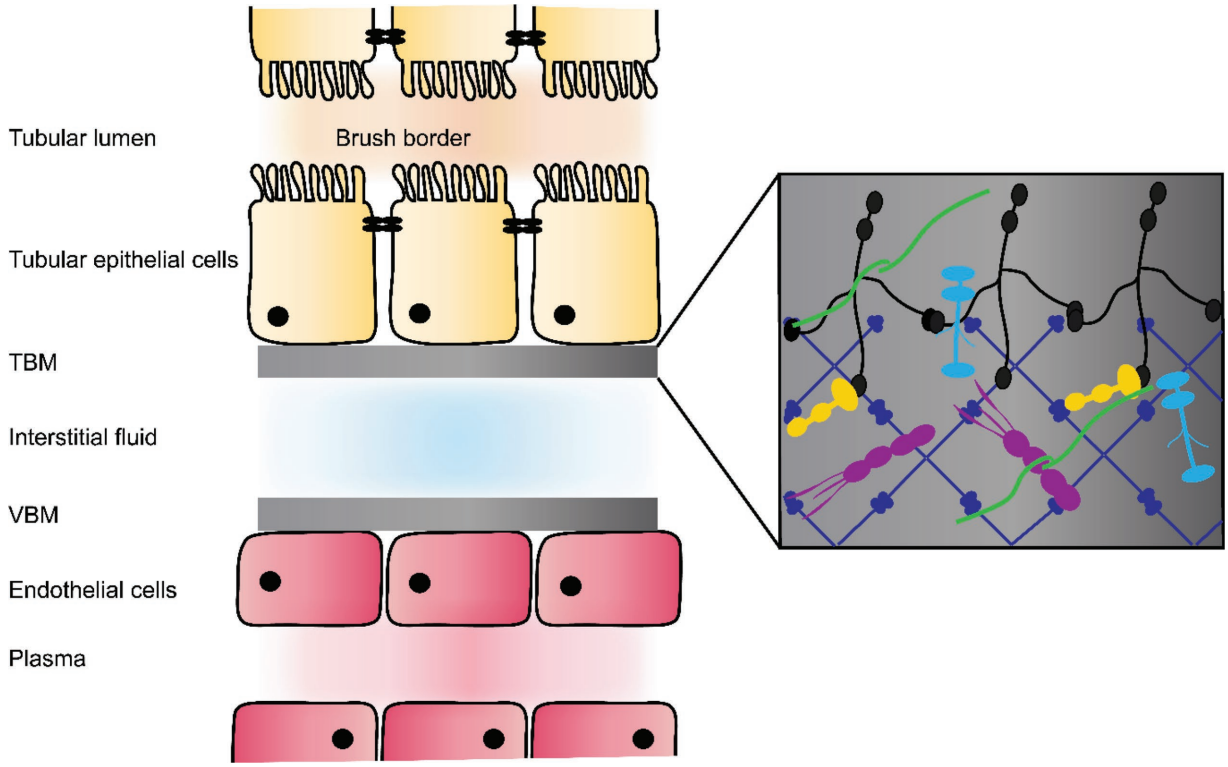

Figure 2. Schematic overview of location of the BM in the renal proximal tubule (TBM) and vasculature (VBM). Enlarged is a schematic drawing of the organized network of the main TBM and VBM components.

laminin-10 ($\alpha5\beta1\gamma1$) are found in the TBM.[16] The vital role of laminin has been confirmed by studies in laminin $\alpha4$-null mutant mice, which developed severe chronic kidney disease because of their mutation.[29] Furthermore, laminin ($\alpha1$) deficient mice showed severe kidney impairment, supporting the important role of laminin.[30]

Integrins, transmembrane receptors, bind to laminin and collagen and facilitate binding of cells to the BM. Integrins are heterodimers composed of an α and β subunit connected by covalent bonds. The $\alpha1\beta1$ and $\alpha2\beta1$ integrins interact with collagen polymers, whereas $\alpha3\beta1$ and $\alpha6\beta1$ integrins specifically bind laminins. $\alpha6\beta1$ is specific for laminin-10.[6]

Glycoproteins:

HSPGs: HSPGs are a group of glycoproteins that all contain one or more covalently attached heparan sulfate chain via which they can bind many ligands. They are located at the cell surface and in the ECM, including the BM. HSPGs can be divided into three groups, (1) membrane HSPGs, (2) secreted ECM HSPGs, and (3) secretory vesicle proteoglycans. The HSPGs that are present in the BM are perlecan, agrin, and collagen XVIII.[31] HSPGs located in the BM are also referred to as a binding reservoir for growth factors that can assist in creating a stable gradient with growth factors, such as vascular endothelial growth factors (VEGFs) for endothelial cells and fibroblast growth factors (FGFs) for epithelial cells. Additionally, HSPGs provide resistance to compression due to their highly hydrated nature.[9,32] Furthermore, HSPGs are filling the space between the meshwork formed of laminin and type IV collagen, and have a major role in creating the charge-selective barrier of the kidney tubule because of their negatively charged heparin sulfate side chains.[33] Besides the HSPGs that are present in the BM, there are also HSPGs that are located at the cell surface that can bind to integrins to facilitate cell attachment to the BM, as well as offering cell–cell interactions.[31] Van den Heuvel et al. were able to isolate HSPGs from human TBM, and found that HSPG accounted for ≈1% of dry weight of TBM.[34]

The major HSPG is agrin (Figure 1), which is present in several isoforms and in many BMs. In the kidney tubule a truncated isoform of agrin that lacks (part of) the C-terminus is predominately present.[35,36] Agrin is involved in the development of neuromuscular junction, which takes place during embryogenesis.[36] Additionally, it may also play a role in renal filtration and interactions between cells and BM.[37]

Perlecan was the first HSPG to be isolated and characterized from the BM.[38–40] The 467 kD core of perlecan is connected to five functional domains of which three are heparin sulfate chains. Perlecan can bind and interact with growth factors and thereby controls cell signaling.[6]

Deletion of either agrin, perlecan or both in the GBM did not result in a loss of the BM structure, which indicates that HSPGs, in contrast to laminin and collagen IV, do not play a pivotal role in the structural arrangement of the BM.[41]

Nidogens: Nidogens (nidogen-1 (NID1) and nidogen-2 (NID2)), formerly known as entactins, are sulfated monomeric glycoproteins that are located in BMs. Nidogens (150–200 kDa) consist of three globular domains (G1, G2, and G3) and are thought to play an important role in the assembly of the BM during development. NID1 and NID2 have a high affinity for

both laminin-1 and collagen type IV and act as the linking molecule between the collagen type IV meshwork and the laminin network.[42–44]

Even though nidogens play an important role in the BM, it seems that they are not crucial for the localization of several BM components (laminin-1, collagen type IV, and perlecan). Mice knockout for either the NID1 or NID2 gene did not show any alterations in the distribution of laminin-1, perlecan, and collagen type IV. Also, mice deficient in both NID1 and NID2 genes, showed similar distributions for laminin-1, perlecan, and collagen type IV. In these double-knockout mice, however, the BM was sometimes thickened.[45]

Hyaluronic Acid: Hyaluronic acid (HA), also called hyaluronan, is a nonsulfated glycosaminoglycan that is abundant in the ECM. HA is important during the embryonic development of the kidney, as it modulates branching morphogenesis.[46] Additionally, HA plays an important role in processes such as angiogenesis and tissue organization.[47] HA is also a promising polymer for the design of materials for tissue engineering applications as it is nonimmunogenic and widely available.[48] In the mature kidney, HA is only present in the interstitium of the renal papilla, but not in the TBM.

Fibronectins: Although its role and presence in the BM have been questioned by several researchers, fibronectin is often stated to be abundant in plasma.[49] While recent literature on the exact role and presence of fibronectin is missing, it is sometimes used as coating agent for tissue culture materials.[50] Fibronectin is a glycoprotein with a large molecular weight (440 kDa) consisting of two nearly identical disulfide bound polypeptides. Many researchers state that fibronectin is located in the mesangium, while other researchers have reached the conclusion that fibronectin must be located in the BM.[17,51–55]

Madri et al. found in an ultrastructural localization of the murine renal BM that fibronectin is mainly located in the mesangial matrix.[28] They were not able to find BM localization for fibronectin. Nevertheless, 20 years later, fibronectin was found in the TBM using high-resolution ultrastructural microscopy.[56]

One of the suggested roles for fibronectin is that it mediates the cell adhesion of mesenchymal cells to collagenous, as well as noncollagenous substrates.[56–58] Additionally, it has been associated with tissue repair after inflammation, functioning as a protein scaffold.[59]

2.1.2. Biophysical Properties

Chemical cues within the BM control cell behavior, but there are also physical cues, such as BM thickness and tissue stiffness, that can influence cell behavior and functioning of the BM itself in many ways. Limited studies have characterized the properties of the epithelial and endothelial BMs, and some results are conflicting (**Table 2**).

Ogawa et al. looked into the TBM using electron microscopy, which revealed that the TBM consists of a fine meshwork structure consisting of fibrils created by collagen IV and laminin.[56] These fibrils form small pores with variable sizes, ranging from 3.3 ± 0.5 nm (short-pore diameter) to 3.9 ± 0.6 nm (long-pore diameter). In the distal tubule, these pores are bigger, while the GBM consists of smaller pores. Whereas the fibril meshwork works as a size selective barrier, the HSPGs create a charge-selective barrier because of their negatively charged heparin sulfate chains.[56]

TBM Thickness: The thickness of all BMs throughout the human body ranges from 100 nm to over 10 µm, and their morphology and composition change with age.[60] The TBM is not a solid membrane, but it is composed of fibrils sized 3–8 nm.[61–63] In rats, the thickness of the TBM ranges from 80 to 100 nm depending on the position within the tubule, their proximal TBM is the thickest. The BM of the loop of Henle and the distal segment in rats are thinner (<80 nm).[13] In humans, the TBM width ranges from ≈360 to 670 nm (Table 2).[64,65]

TBM Stiffness: Tissue stiffness is mostly determined by the composition of the ECM. The degree of tissue stiffness will greatly influence proliferation and differentiation of cells. Many tissues are relatively soft, ranging in elastic modulus from 100 Pa to 100 kPa.[66] The Young's modulus (YM), also called elastic modulus, is used to describe the tensile or compression elasticity of any object reflecting its ability to return to its original shape after a force is applied. When looking for the YM of TBMs within the literature, values ranging from 3 to 10 MPa have been reported.[67,68] However, these values differ greatly from the YM that has been reported for the whole kidney.[13,69,70] In Table 2, an overview is given on the YM found in various segments of the kidneys of diverse species. Variations can be explained by the different origin of the tissues used and techniques used for measuring. Recently, Beamish et al. investigated the effect of ECM stiffness on the ability of renal proximal tubular cells to form an epithelial-like structure.[71] They observed that an increase in substrate stiffness resulted in better renal proximal tubule epithelial cells (RPTEC) spreading as well as their proliferation. Additionally, when culturing RPTEC for a longer time the stiffness of the hydrogel promoted the formation of epithelial monolayers that were more complete with tight junctions, cell polarity, and an organized BM. However, Chen and co-workers showed that mouse PTECs preserve tubular-like structures grown on soft (≈60 Pa) matrigel, whereas the cells did not differentiate on hardening (≈1200 Pa) matrigel.[72] Also, in various disease models an increase in TBM stiffness is seen with progression of disease.[69,73]

3. The Role of the BM in Vascularization and its Major Components

The VBM is located between the endothelial cell lining and the pericytes that make up the outer wall of the blood vessel. Pericytes are contractile, mural cells embedded in the VBM.[78,79] The VBM separates various tissue compartments and stabilizes the vascular tubule. Additionally, it provides cues to the endothelial cells through its chemical components. The VBM is a complex meshwork that consists of pores and fibers.[77] While the VBM functions as a substratum for the endothelial cells, it is also a selective barrier and regulates the survival of the cells. Changes in the thickness of the VBM affect the physical features and thereby the cell behavior. Therefore, it is important to know the exact dimensions of the physiological BM, to be able to mimic such a structure for tissue engineering strategies.

Table 2. Overview of the literature on biophysical properties of TBM, VBM, and renal ECM measured in various species.

	Species, tissue	Mechanical properties Young's modulus (YM) Shear modulus (SM)	BM thickness	BM pore size/fiber size	Method	References
Kidney	Human, TBM		558 ± 116 nm		Percutaneous biopsy, light, and electron microscopy	[64]
	Human, TBM		399.7 ± 33.72 nm		Biopsy, light, and electron microscopy	[65]
	Human, kidney	Axial phase image SM: 4.12 ± 0.24 kPa Coronal oblique image SM: 4.32 ± 0.59 kPa Sinus: 6.78 kPa ± 0.10 kPa Medulla: 5.46 ± 0.48 kPa Cortex: 4.35 ± 0.32 kPa			Magnetic resonance elastography (MRE)	[70]
	Ex vivo: Mouse, TBM	Low-strain (10%) YM: 284 ± 90 kPa High-strain (30%–40%) YM: 3230 ± 356 kPa			Mouse kidneys fixed and analyzed using transmission electron microscopy (TEM)	[8]
	Ex vivo: Rabbit, TBM	YM: Proximal straight tubule BM: 10 ± 1 MPa YM: Proximal convoluted tubule BM: 8 ± 2 MPa	0.26 µm in intact perfused rabbit proximal and collecting tubules		Rabbit kidneys were analyzed using an inverted microscope	[67]
	In vivo: Rabbit, kidney cortex, medulla and sinus	YM: Kidney Cortex: 16.34 ± 1.01 kPa Medulla: 13.71 ± 1.16 kPa Sinus: 12.61 ± 0.84 kPa			Shear wave elastography (SWE)	[69]
	In vivo: Rat, kidney	SM: Renal cortex: 3.87 ± 0.83 kPa			MRE	[73]
	Ex vivo: Rat, TBM			Short pore: 3.3 ± 0.5 nm Long pore: 3.9 ± 0.6 nm Fibrils: 1.6 ± 0.3 nm	Rat kidneys were fixed and analyzed using TEM	[56]
	In vivo: Swine, kidney	SM: Cortex: 6.0 ± 0.7 kPa Medulla: 6.5 ± 0.6 kPa			MRE	[74]
Vasculature	Human, Descemet's BM	Descemet's BM: 50 ± 17.8 kPa		Descemet's BM: 38 nm (pore)	AFM	[75]
	Rabbit, Corneal endothelium	Endothelium: 4.1 ± 1.7 kPa Descemet's BM: 11.7 ± 7.4 kPa			AFM	[76]
	Ex vivo: Rhesus Macaque, VBM Descending aorta (DA) Left common carotid (LCC) Left saphenous vein (LSV) Inferior vena cava (IVC)		DA: 506 ± 14 nm LCC: 319 ± 14 nm LSV: 112 ± 8.2 nm IVC: 286 ± 8.2 nm	DA: 59 ± 4.5 nm/31 ± 1 nm LCC: 63 ± 6 nm/30 ± 2 nm LSV: 38 ± 2 nm/27 ± 1 nm IVC: 49 ± 2 nm/24 ± 0.6 nm	TEM, scanning electron microscopy (SEM)	[77]

Similar to the TBM, the 3D networks of laminin and collagen IV within the VBM are formed independently and connected via HPSGs (perlecan) and nidogens.[80] Nidogens bind both collagen type IV and laminin and thereby bridge the two networks.[42] The various components of the VBM can bind many growth factors and cytokines and thereby function as a storage depot of growth factors for the cells. Growth factors and cytokines such as VEGF-A can be released from the VBM to stimulate differentiation and proliferation of cells.[81] VEGF stimulates both vasculogenesis and angiogenesis, primarily via interactions with receptors located in the endothelial cells (VEGFR1 and –R2).

3.1. Collagen Type IV

Collagen IV is the major component of the VBM and crucial for membrane stability, as well as the structural integrity of small vessels.[24] Similar to the TBM the collagen IV isoform present in the VBM is α1(IV)/α2(IV). Via its eight cysteine residues,

collagen IV can make intra and intermolecular disulfide bonds, which aids the stabilization of the collagen IV network.

3.2. Laminins

The major isoforms present in the vessel wall are laminin α4 and α5 that bind with laminin β1 and γ1 to form laminin-8 and laminin-10, respectively. In many tissues laminins shift in specificity over time, as laminin-5 is expressed by endothelial cells during early development and angiogenesis. In mature vasculature it shifts from laminin-5 to laminin-10.[6,82] Without laminin α4 microvessel maturation is impaired in mice, indicating that laminin α4 plays a central role in the microvessel development.[83] Endothelial cells are anchored via endothelial β1 and β3 integrins to the laminins in the VBM.[80,84–87]

3.3. Glycoproteins

3.3.1. HSPGs

Perlecan and agrin are the main HSPGs present in the VBM; however, certain HSPGs can also be located at the cell surface. Perlecan can bind to growth factors from the FGF family, and is a regulator of neovascularization as binding to FGF leads to enhanced angiogenesis.[88,89] In zebrafish, it was found that perlecan is involved in developmental angiogenesis by interfering with VEGF-VEGFR2 signaling events.[90] The main roles of HSPGs include adhesion of cells to the BM, and promoting cell proliferation and differentiation.[91–93]

3.3.2. Nidogens

Both NID1 and NID2 are present in the VBM and seem to be complementary in function, NID2 is enriched in endothelial BMs.[94,95] Nidogens conserve the structural integrity of the VBM via connecting BM components laminin and collagen type IV via domain-specific interactions.[42] While nidogens are involved in connecting the laminin and collagen IV networks, nidogens are not crucial for the formation of the BM. Mice mutant for either nidogen-1 or nidogen-2 developed normal endothelial and epithelial BMs and no abnormalities were observed in the vasculature, but they did show neurologic deficits.[95–97] One of the reasons might be that in mice with a deficiency for one of the nidogens, the other nidogen compensates for its loss in function.[98] Nevertheless, deletion of both the nidogen-1 and nidogen-2 gene in mice resulted in perinatal lethality due to incomplete lung development, as well as cardiac defects.[99] Besides their role in connecting laminin and collagen type IV, nidogens can also interact with the integrin receptors on cells to mediate cell adhesion to the BM.

3.4. Other Components

In addition to the major components, the VBM contains some minor, vessel specific, components including BM40 (osteonectin), fibulins-1 and -2, collagen VIII, XV, and XVIII, and thrombospondins-1 and -2.[100] Collagen XV and XVIII are both expressed in capillary BMs, but in the fenestrated endothelium of the glomerulus only type XVIII is found to be present.[101] Whether this also holds true for the TBM is unknown, although it seems that in many specialized vessels only type XVIII is present including in liver sinusoid, splenic sinusoid, and alveolar capillaries. The differential distribution of collagen XV and XVIII might be an initiator of the different functions of the various capillaries.

3.5. Biophysical Properties of VBM

While several approaches have been made toward engineering of vascular tissue, the biophysical properties of the vasculature are not always incorporated in the design of the biofabricated vessels. Since the topography and compliance of the vessel can largely influence its nature, a better understanding of the biophysical properties of the VBM might improve the tissue engineered vessels.[102,103]

3.5.1. VBM Thickness

Thickness of the VBM ranges from the nano to submicrometer scale but varies significantly between vascular tissues, which indicates that membrane thickness can influence biophysical properties such as compliance and topography.[77] The data available on VBM topography are limited to the corneal VBM and rhesus macaque vessels VBM (Table 2). The data shown on the VBM indicate ≈100–500 nm in rhesus macaque.[77] However, it is not clear whether the dimensions for the renal tubular VBM are in the same range. Whole organ engineering involves the use of various bioprinting techniques that are, depending on the method used, often limited by their printing resolution. Hence, much higher printing resolutions might be needed to obtain a physiologically relevant model. Yet, most literature on VBM thickness originates from animal studies, which is not directly translatable to humans as VBM thickness will be related to the size of the animal and the blood pressure it needs to endure.

3.5.2. VBM Stiffness

Data available on the stiffness of the VBM are limited to Descemet's BM, which is the membrane underlying the endothelial cells in the cornea (Table 2). In two independent studies the Young's modulus of Descemet's BM was measured using atomic force microscopy (AFM), whereby values were found ranging from 11.7 ± 7.4 kPa in rabbits to 50 ± 17.8 kPa in humans.[75,76] Like the thickness of the membrane, also the stiffness will greatly depend on the size of the animal, as well as the blood pressure it needs to endure. More accurate data are needed to have a clear overview of the elasticity and strength of the BMs, to be able to mimic those properties in any synthetic or biologic BM.

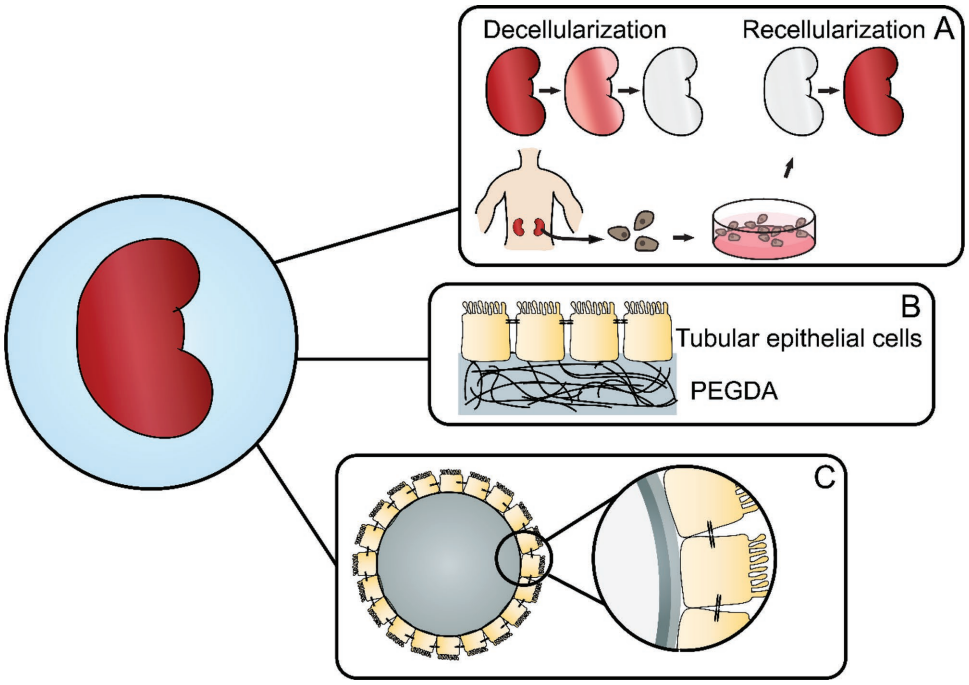

Figure 3. Schematic overview of applications of membrane technology in kidney research. A) De- and re-cellularization of native kidney scaffolds. B) Hydrogels for tissue engineering applications. C) Hollow fiber membranes as scaffolds for kidney cells.

3.6. Connection between VBM and TBM

In the nephron, there is a constant exchange of molecules between the renal epithelial cells and the endothelial cells, as waste products and endogenous and exogenous solutes are filtered from the blood into the tubular lumen and form the ultrafiltrate. One can imagine that it is of vital importance that peritubular capillaries and the renal proximal tubules with their BMs are in close proximity to allow efficient and effective exchange. For the GBM, it has been shown that it firmly connects to the VBM and even can fuse to form one single BM between the capillaries and the podocytes.[104,105] The exact distance or fusion of/between the proximal TBM and VBM remains unknown.

4. Current and Novel Applications of Membrane Technology in Kidney Research

The most recent strategies for tissue engineering of (parts of the) kidneys include 3D-bioprinting and molding hydrogels, as well as decellularization of organs for generation of suitable (native) scaffolds. For this, researchers have used BM components to improve the quality of engineered ECM of the scaffolds.[48]

4.1. Decellularization and Recellularization of Kidney Scaffolds

Decellularization is a method whereby all cellular components are removed from the organ matrix by perfusing the vasculature of the organ with a range of detergents such as Triton X-100 and sodium dodecyl sulfate (SDS), enzymes or cell lysing solutions.[106–109] What is left after decellularization is the ECM of the organ which is still specific in terms of morphology and certain biological cues. These organ scaffolds can be recellularized to create a new organized tissue (**Figure 3**A). Several research groups have successfully shown the decellularization of rat kidneys.[106,110–113] Ross et al. were the first to report the complete recellularization of a rat kidney scaffold.[110] Bonandrini et al. showed that the expression of the ECM proteins such as collagen IV, laminin, and fibronectin in a decellularized rat kidney were similar to that of a native kidney, confirming the ECM remains intact.[111] Caralt et al. compared the different protocols for decellularization and found that the best results are obtained with Triton X-100 and SDS, where the cells are effectively removed, but the ECM scaffold and the ECM growth factors remain preserved.[112] Next to rats, also the kidneys of rhesus monkeys, pigs, and humans have been used for decellularization.[114–117] Human kidneys are of course the best source of renal ECM, as differences in ECM composition and architecture of the scaffolds between species have been reported (Table 2). To this end, human kidneys discarded from transplantation have been used.[118–121]

With decellularization, all native cells are removed as well as immunogenic proteins present in the scaffold. One of the major advantages of this strategy is that the ECM with its 3D structure and composition remains intact, while in other biofabrication techniques it is a challenge to mimic the ECM with all its tissue-specific structural and chemical components.[107] The most challenging part, however, is the recellularization of the scaffold because of the complexity of the kidney that consists of over 20 cell types. Therefore, complete repopulation of the scaffold and its functional restoration have as of yet

not been successful. There are many more difficulties in the recellularization process, and often incorporation of certain growth factors or bioactive molecules are needed to enhance the function of the created tissue.[122,123] But also technical challenges are a reason for the unsuccessful recellularization of kidney scaffolds. De- and recellularized scaffolds that are implanted in vivo often lead to cell clotting within the scaffolds, forming a major risk for thrombosis.[115] Whether this is inherent to the use of synthetic ECM components needs to be thoroughly investigated. In addition, it seems that although most of the structure and composition of the scaffolds remain intact after decellularization, depending on the method of decellularization, the compliance, Young's Modulus, and stress–strain curves can decrease or increase, as reviewed by Boccafoschi et al.[124–127]

Decellularization strategies are not limited to kidney research, a similar approach has been used for vascular tissues whereby intact human greater saphenous vein specimens were decellularized and used for vascular tissue engineering.[128–131] In canines, decellularized veins were used as vascular grafts in vivo, which exhibited satisfactory strength and supported cell repopulation after 8 weeks of arterial flow.[128] Zhao et al. created tissue-engineered blood vessels (TEBVs) using decellularized ovine carotid arteries.[130] After decellularization the scaffolds were seeded with autologous cells and interposed into the carotid arteries in an ovine host model. The TEBVs were mechanically stable for 5 months in vivo and showed presence of endothelium, smooth muscle, collagen, and elastin.

4.2. Hollow Fiber Membranes

To improve current renal replacement treatment strategies such as hemo and peritoneal dialysis, the development of a bioartificial kidney device (BAK) has sparked considerable interest during the past decades. Whereas in current RRTs synthetic membranes are used to filter the blood from toxins, in a BAK device these membranes are covered with renal cell monolayers that increase the efficacy of the membranes (Figure 3C).

Researchers from our group developed living membranes by culturing conditionally immortalized proximal tubule epithelial cells (ciPTEC) on biofunctionalized MicroPES (polyethersulfone) hollow fiber membranes (HFMs).[132] The ciPTECs were able to form a functional monolayer on the HFM with zonula occludens-1 (ZO-1) protein expression, and organic cation transporter 2 (OCT2) activity. The HFMs were coated with combinations of laminin, gelatin, matrigel, collagen IV, and L-3,4,dihydroxydiphenylalanine (L-DOPA) coatings, prior to seeding of ciPTEC.[133] The coating procedures were established first by other groups in search for BAK materials and coatings for bioreactor units.[134–136] Finally, most promising results were obtained with a combination of collagen IV and L-DOPA.[132,135] More recent, Jansen et al. also showed that living membranes obtained with the double-coating strategy were able to mediate the active transport of protein-bound uremic metabolites.[137] These results are a promising step forward and demonstrate that also other materials mimicking the ECM can serve as a base for kidney cells to grow and function. Also, the researchers have shown that using a BM component such as collagen type IV to coat the matrix can improve cell adhesion and proliferation. The next step is to further upscale this device so that it can eventually be used in the clinic.[138]

4.3. Hydrogels as Extracellular Matrix

The use of hydrogels as biomaterials in tissue engineering has significantly increased over the past decades. Hydrogel matrices consist of (a combination of) biological and synthetic polymers that are stably connected via physical (noncovalent) or chemical (covalent) crosslinks.[139] Because of their biocompatibility and easy modifications in biochemical and mechanical properties, these 3D polymer networks are often used as artificial ECM or scaffolds for cells (Figure 3B). As their name implies, hydrogels can retain water, called the swollen state of a hydrogel. Certain hydrogels can be used in combination with 3D-printing techniques to create free standing tubes, or other shapes. Often techniques such as molding are used as well, to create channels within a hydrogel. The mechanical properties of hydrogels can easily be tuned by changing, e.g., the crosslinking density or the polymer concentration. One benefit of hydrogels is that they can easily be combined with microfluidics, whereby the hydrogel functions as the ECM within the microfluidic chip and flow can easily be controlled.[140] In physiology, the VBM has to endure a lot of shear stress because of the circulating blood volume, for which stiff synthetic hydrogels can be used to support the physiological shear stress levels. However, when a gel is too stiff, cell growth as well as migration of growth factors and nutrients can be limited.

A poly(ethylene glycol) (PEG) based hydrogel has been used to study the effect of the mechanical properties of the substrate on the proliferation of renal cells.[71] Increased hydrogel stiffness promoted monolayer formation of renal epithelial cells when cultured for up to 2 weeks, suggesting that ECM stiffness can regulate monolayer formation.

Kolesky et al. created thick (>1 cm) vascularized tissues whereby parenchyma, stroma, and endothelium were integrated into a single thick tissue. Using several cell types in a customized hydrogel ECM, the vascularized tissues were viable for over 6 weeks. Their matrix is based on a combination of gelatin, fibrinogen, transglutaminase, calcium chloride, and thrombin, which allows for cell adherence and monolayer formation.[141]

The same group has developed bioprinting approaches for renal proximal tubules using a similar gelatin-based matrix.[142] For this, they used a sacrificial bioprinting method using Pluronic-127 to create a perfusable channel in the gel-matrix, subsequently seeded with proximal tubule epithelial cells. Within this setup, the cells formed a tight monolayer with a functional barrier that was assessed using fluorescein isothiocyanate (FITC)-labeled inulin perfusion; the cells were cultured for over 2 months. Furthermore, in this setup the tubule cells exhibited superior albumin uptake function compared to a 2D control.

Remarkably, the components that are used in the hydrogels mentioned here (e.g., PEG, gelatin, fibrinogen, and thrombin) are not the main components of the native BM. While hydrogel components partly mimic physical characteristics of the BM by exerting similar structures or mechanical properties, they lack

chemical components that can trigger cell proliferation and differentiation. Hence, there is a potential for improving hydrogel composition for mimicking the BM. Su et al. have used hydrogels derived from tissue and organ-specific decellularized ECM for culturing of glomerular endothelial cells.[143] While the glomerular endothelial cells showed high viability and proliferation, their gene expression for relevant genes was decreased compared to cells encapsulated within hydrogels that were composed of collagen I. Further research is needed to investigate the benefit of ECM-derived hydrogels, or maybe a combination of ECM-derived hydrogels and hydrogels such as PEG for tissue engineering.

4.4. Biofunctionalized Polymers

Mollet et al. established BM mimics made from ureidopyrimidinone-functionalized polymer and bioactive peptides by electrospinning.[21] Cells were seeded on the BM mimics in a bioreactor. They were able to show that human kidney-2 epithelial cells were able to form a polarized monolayer on the BM mimics, and show modulation of gene expression and important membrane transporter proteins. Biofunctionalized polymers are used to mimic the BM via (melt) electrospinning, whereby thin fibers are spun to create a meshwork that finally functions as the scaffold.[144] While the polymers itself might lack some of the biochemical properties of the BM, they can largely mimic the biophysical properties by optimizing the size of the fibers to create a meshwork like the ones formed by collagen IV and laminin. Scaffolds designed using melt electrospinning writing have shown to support cell attachment, proliferation, ECM formation, and infiltration. By coating biofunctionalized polymers with collagen IV or laminin, the polymers could mimic the BM to an even higher extent.

5. Conclusions

The BM plays an important role in organogenesis, as it stimulates cell growth, cell attachment, and proliferation, it enhances angiogenesis, and serves as a growth factor depot for the cells attached to the BM. Although the major components of the BM have been discovered, knowledge on the exact physical properties remains unclear. This opens up opportunities for researchers to further investigate the properties of the BM, and to be able to mimic their role in bioengineered kidneys or related structures.

Many approaches have been made to mimic the BM using various strategies, such as scaffolds obtained from native kidney decellularization, hydrogels, hollow fiber membranes, or biofunctionalized polymers. The major advantage of decellularization is that the ECM, including the BM, remains intact. Creating BM from scratch requires critical steps to take, as it is a very complex matrix. Nevertheless, it is still very difficult to recellularize complex structures such as the kidney, which consist of many different segments and cell types. Other, more simple approaches have been shown to be quite effective and promising, as it appeared possible to mimic the meshwork of the BM using hollow fiber membranes and biofunctionalized polymers, whereby a porous membrane serves as the BM. Additional coatings such as collagen IV can improve cell attachment and proliferation. Using more elaborate compositions of coatings might improve cell behavior as well, as a collagen IV coating only partly resembles the major components of the BM. 3D bioprinting of kidney tubules still seems to be a challenging task, many researchers therefore use molding techniques to create channels within a hydrogel matrix instead.

While we still have a long way to go before we can create a fully functional kidney, the first steps have already been taken by many researchers in the field. When combining hydrogels that can be fine-tuned and enriched with some of the major components of the BM such as collagen IV, laminin, and fibronectin, with biofunctionalized polymers physically mimicking the collagen IV/laminin meshwork, we may be able to create a BM that allows for better cell proliferation and differentiation. A better understanding of the exact composition of the BM and its role in tissue performance will advance the process of engineering functional vascularized kidney tubules, as it will guide us to fabricate scaffolds that are suitable for the growth of functional renal proximal tubule and endothelial cells.

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Conflict of Interest

The authors declare no conflict of interest.

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