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Review Article

Pathophysiology of the Peritoneal Membrane during Peritoneal Dialysis: The Role of Hyaluronan

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During peritoneal dialysis (PD), constant exposure of mesothelial cells to bioincompatible PD solutions results in the denudation of the mesothelial monolayer and impairment of mesothelial cell function. Hyaluronan, a major component of extracellular matrices, is synthesized by mesothelial cells and contributes to remesothelialization, maintenance of cell phenotype, and tissue remodeling and provides structural support to the peritoneal membrane. Chronic peritoneal inflammation is observed in long-term PD patients and is associated with increased hyaluronan synthesis. During inflammation, depolymerization of hyaluronan may occur with the generation of hyaluronan fragments. In contrast to native hyaluronan which offers a protective role to the peritoneum, hyaluronan fragments exacerbate inflammatory and fibrotic processes and therefore assist in the destruction of the tissue. This paper will discuss the contribution of mesothelial cells to peritoneal membrane alterations that are induced by PD and the putative role of hyaluronan in these processes.

1. Introduction

Peritoneal dialysis (PD) is an effective form of renal replacement therapy that is currently used by approximately 11% of the total global dialysis population. Although PD has greatly improved the quality of life in renal patients, a major disadvantage of this treatment is that PD solutions are bioincompatible since they contain elevated concentrations of glucose to provide the osmotic drive, a low pH to prevent glucose caramelization during heat sterilization and lactate to correct metabolic acidosis. Heat sterilization of glucose-based PD solutions and storage can give rise to the formation of glucose degradation products (GDPs). Besides their direct toxic effect on mesothelial cells [1], GDPs promote the formation of advanced glycation end products [2]. These constituents have been shown to provoke peritoneal inflammation and injury in mesothelial cells, resulting in structural changes in the peritoneal membrane and progressive loss of peritoneal functions [3–5]. Studies have demonstrated that many patients on long-term PD exhibit reduplication of the basal lamina, increased synthesis and deposition of matrix proteins within the submesothelium, and progressive subendothelial hyalinization, with narrowing or obliteration of the vascular lumen [6, 7]. These structural changes are exacerbated by episodes of peritonitis and are associated with a loss of ultrafiltration and solute clearance that leads to technique failure and unfavorable clinical outcomes. Specifically how these changes in the peritoneal membrane are regulated remains to be fully elucidated.

Hyaluronan is a large glycosaminoglycan that is constitutively synthesized by mesothelial cells. We and others have demonstrated that hyaluronan plays a crucial role in the maintenance of mesothelial cell morphology and remesothelialization [8, 9]. While the mechanisms through which hyaluronan participates in peritoneal homeostasis and pathological processes are being investigated, there is accumulating data to show that the hyaluronan molecule can exert either protective or potentially destructive effects depending on its molecular weight, local concentration, and tissue distribution. This paper will focus on the putative role

of hyaluronan in peritoneal homeostasis and how alterations in hyaluronan synthesis induced by PD can contribute to peritoneal inflammation, EMT, and fibrosis.

2. Peritoneal Mesothelial Cells

The peritoneal membrane consists of a monolayer of mesothelial cells underneath which contains the submesothelium, fibroblasts, collagen fibrils, and capillaries [10]. Peritoneal mesothelial cells are specialized epithelial cells that line the peritoneal cavity. Although previously thought to function solely as a lubricating, nonadhesive surface to facilitate intracoelomic movement, there is now compelling evidence to demonstrate that peritoneal mesothelial cells are not passive cells but play critical roles in peritoneal homeostasis, maintenance of peritoneal membrane integrity, fluid, and solute transport, peritoneal inflammation, and wound healing. Mesothelial cells regulate peritoneal inflammation and tissue remodeling by virtue of their ability to synthesize a plethora of cytokines, growth factors, and matrix proteins [11–14]. Mesothelial cells also synthesize glycosaminoglycans and proteoglycans such as hyaluronan, decorin, syndecan-1, and perlecan that endow the mesothelium with a protective glycocalyx and selective permeability property [15–24].

Although mesothelial cells are derived from the mesoderm, they possess many features of epithelial cells that include a polygonal, cobblestone morphology with surface microvilli, are situated on a basement membrane, express cytokeratin, and have the ability to form a polarized monolayer that permits translocation of molecules to either the apical or basolateral aspect of the cells [25–29]. Cell polarity is essential to maintain normal mesothelial cell function and occurs upon cell-cell contact. The precise distribution of proteins to the apical or basolateral aspect of the cell is critical for cell signaling and interaction with their microenvironment. With regard to their mesenchymal characteristics, mesothelial cells can express vimentin, desmin, and α-smooth muscle actin, the latter induced during cell activation [25, 29–31]. Mesothelial cells are connected by intercellular junctions (tight junctions, gap junctions, adherens junctions, and desmosomes) that contribute to the establishment and maintenance of a continuous mesothelial monolayer [32]. Mesothelial cells have been shown to express ZO-1, occluding, and claudin-1 [33, 34]. Reduced expression of adherens junctions during pathologic conditions or inflammatory processes is associated with a collapse of cell-cell communication and cell-matrix interaction resulting in the denudation of the mesothelium, a process that is often observed during PD [33, 34].

Since an in-depth discussion on mesothelial cell structure and function is beyond the scope of this paper, the reader is referred to the related reviews [26, 27, 35–37].

3. Hyaluronan

Hyaluronan is a negatively charged, linear polysaccharide that is widely distributed in epithelial, connective, and neural tissues [38, 39]. Unlike other glycosaminoglycans, it is neither sulfated nor is it attached to a protein core. It is composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine and is synthesized on the inner surface of plasma membranes by uridine diphosphate glucose dehydrogenase and one of three related hyaluronan synthases (HAS), namely, HAS I, II, or III [40]. These isoenzymes have a 55–70% homology, and HAS II is critical for animal survival [41]. HAS I is the least active of the HAS isoenzymes and together with HAS II controls the synthesis of high molecular weight (HMW) or native hyaluronan. HAS II is more active than HAS I, and its upregulation is implicated in wound healing, inflammation, and tissue growth. Of the 3 isozymes, HAS III is the most active and drives the synthesis of low molecular weight (LMW) hyaluronan. Given that HAS I, II, and III expressions are differentially regulated by cytokines and growth factors [42], it is plausible to suggest that depending on the inciting factor, one or more HAS isoforms may be upregulated to induce hyaluronan synthesis of different molecular weights and abundance.

Under physiologic conditions, hyaluronan is synthesized as a macromolecule with a molecular weight in excess of 10^6 Da [43]. Following its synthesis, hyaluronan is directed to the cell surface where it interacts with its receptor CD44 or it is assembled into the pericellular matrix (glycocalyx) or extracellular matrix (ECM). The regulation of hyaluronan translocation to the different compartments of the cell is currently unknown, but it is possible that mechanisms used to export other polysaccharides may be involved such as the adenosine-5′-triphosphate-binding cassette system. Despite its simple structure, hyaluronan is a multifaceted molecule that contributes to the structural integrity of tissues, maintains water balance, assists in the distribution and transport of plasma proteins, promotes cell quiescent, decreases fibrotic mediators, and possesses anti-inflammatory properties [44–46]. Due to its ability to regulate cell proliferation, migration and phenotype, and cell-cell communication, hyaluronan also plays a pivotal role in metastasis and tumorigenesis [47–49]. Hyaluronan can influence physiologic cell behavior through three mechanisms. Firstly, hyaluronan can form tertiary structures in aqueous solutions and interact with water molecules resulting in the production of a hydrated environment with high viscosity and elasticity that allows cells to divide and migrate [50]. Secondly, hyaluronan contributes to the assembly and structural integrity of pericellular matrices, which influence cell shape during cell division, movement, and morphogenesis [51]. Thirdly, hyaluronan can influence cell behavior through its interaction with its cell surface receptors, namely, CD44 or receptor for hyaluronan-mediated motility, and initiate intracellular signaling [52, 53].

HMW hyaluronan undergo constant turnover during the daily maintenance of basement membranes, and its degradation into small, nonbiologically active fragments is rapidly removed from the body by the liver. In chronic inflammation, elevated serum levels of hyaluronan and its deposition at sites of injury are often observed. The accumulation of hyaluronan in tissues has been shown to exacerbate inflammatory processes. Fragmentation of ECM components often occurs during tissue injury, and these fragments possess functional properties that are distinct from

Table 1: Functions of native hyaluronan and its fragments during tissue homeostasis and inflammation.

HMW hyaluronan	LMW hyaluronan
Contributes to the protective role of the glycocalyx, acts as a lubricant	Induces chemokine and cytokine secretion by infiltrating, mesothelial, renal tubular epithelial and endothelial cells
Transportation and distribution of plasma proteins	Induces phosphorylation of signaling pathways, for example, MAPK
Contributes to water balance and regulation of tissue hydration	Induces cell migration, for example, tumor cells
Contributes to tissue integrity and maintenance of epithelial cell phenotype	Induces cell proliferation in chondrocytes, endothelial cells, and fibroblasts
Protects against tissue damage by scavenging free radicals	Activates NFκB
Protects against apoptosis	Induces nitric oxide synthase
Antiangiogenic	Promotes angiogenesis
Inhibits phagocytosis by monocytes and macrophages	Increases matrix protein synthesis, for example, collagen type I
Anti-inflammatory, can inhibit activation of inflammatory cells	Increases transcription of matrix metalloproteinases
Promotes cell quiescence	Suppresses cell death and apoptosis in cell culture
Immunosuppressive (prevents ligand binding to surface receptors)	Induces heat-shock protein expression

LMW hyaluronan: ranges from 4 to 40 saccharide units.

their parent molecule [46]. The clearance of ECM fragments is therefore imperative for the resolution of tissue injury. Independent researchers have suggested that LMW hyaluronan may deposit in inflamed tissues consequent to their *de novo* synthesis or through the depolymerization of native hyaluronan following increased activity of hyaluronidase or reactive oxygen species [46, 54, 55]. Unlike native hyaluronan, hyaluronan fragments have been shown to promote angiogenesis, induce multiple signaling cascades, and increase cell proliferation, cytokine secretion, matrix metalloproteinase (MMP) activity, and matrix protein synthesis in murine models of lung disease or cultured mesothelial and endothelial cells, keratinocytes, macrophages, and dendritic cells [46, 56–62]. Proinflammatory cytokines and profibrotic growth factors have been shown to increase synthesis of both HMW and LMW hyaluronan in various cell types [63–66]. Table 1 summarizes the distinct roles of HMW and LMW hyaluronan under physiologic and inflammatory conditions. Despite numerous articles highlighting the inflammatory properties of LMW hyaluronan *in vitro* or *ex vivo*, one must take note that reports detailing the actual appearance of LMW hyaluronan in tissues undergoing inflammation are limited [67]. The lack of antibodies that distinguishes between native HA and its fragments may, in part, contribute to this paucity of data, and therefore in-depth biochemical methodologies are warranted to provide evidence of their presence at sites of inflammation and tissue injury.

4. The Role of Hyaluronan in the Maintenance of the Normal Peritoneal Membrane

In the healthy individual, a thin film of fluid is found on the surface of mesothelial cells, which serves as a lubricant for the peritoneal viscera that protects the mesothelial surface from abrasions and adhesions. This fluid constitutes the glycocalyx and contains a number of macromolecules that include lipoproteins, phospholipids, and hyaluronan. The integrity of the glycocalyx is, in part, attributed to the presence of HMW hyaluronan [68, 69]. The glycocalyx contributes to the protective, nonadhesive nature of the mesothelial cell surface and plays an important role in cell-cell contact, tissue integrity and hydration, regulation of inflammation, wound healing, and flow of nutrients and growth factors across the peritoneal membrane [70]. The luminal surface of mesothelial cells is endowed with microvilli [28]. Microvilli entrap water and serous exudates, which protect the delicate surface of mesothelial cells from frictional damage [27]. Microvilli allows mesothelial cells to sense their microenvironment and also functions to entrap bacteria thereby preventing infection. The density of microvilli on regenerating mesothelial cells may vary and is dependent on the anionic charge of the glycocalyx [28]. Recent studies have demonstrated that the presence of hyaluronan in the glycocalyx of MCF-7 cells is essential for the formation of microvilli and that the length of these protrusions is dependent on HAS activity and the rate of hyaluronan synthesis [71]. The removal of hyaluronan from microvilli following treatment with hyaluronidase is accompanied by their retraction from the cell surface [71]. The synthesis of microvilli on mesothelial cells has not been fully investigated, but it is possible that it is regulated by hyaluronan as observed in MCF-7 cells. The presence of microvilli on the surface of mesothelial cells can increase the surface area of the peritoneal membrane available for peritoneal transport from 2 m^2 to 40 m^2. A reduction in the number of microvilli on mesothelial cells would therefore have a profound effect on peritoneal function and transport.

The peritoneal mesothelium provides the first line of defense against bacteria, chemical, or surgical insult. It is therefore essential that following injury and denudation, rapid restoration of the mesothelial monolayer takes place. The mechanism through which the normal mesothelium is restored is controversial and has been suggested to involve centripedal migration of mesothelial cells as observed in the squamous epithelium, exfoliation of healthy mesothelial cells from neighboring sites which settle on the denuded area,

free-floating reserve cells, submesothelial and bone-marrow-derived precursor cells, and macrophage transformation [27, 72–79].

We and others have shown that mesothelial cells synthesize large quantities of hyaluronan that is secreted into their microenvironment or can be found on the mesothelial cell surface as a major constituent of the mesothelial glycocalyx [16–18, 63, 80]. Hyaluronan plays an essential role in peritoneal homeostasis, but changes in hyaluronan synthesis and its interaction with its cell surface receptors can have a profound effect on cell function. Increased hyaluronan synthesis in mesothelial cells is associated with increased cell migration, proliferation, and phenotypic changes [47, 81]. Using an established *in vitro* model of wound healing, we have demonstrated that, following mechanical denudation of the mesothelial monolayer, repopulation of the monolayer is mediated by the migration of mesothelial cells from the leading edge of the wound into the denuded area [8]. This process is accompanied by a loss of cell-cell contact at the wound margin and induction of epithelial-to-mesenchymal transdifferentiation (EMT), a reversible process that bestows upon mesothelial cells a migratory, invasive fibroblastic phenotype and is accompanied by the dissolution of intercellular junctions, loss of cell polarity, reorganization of the cytoskeleton and focal adhesion components, and increased synthesis of matrix proteins and hyaluronan, the latter observed predominantly in migratory, elongated mesothelial cells [8, 82]. The increase in hyaluronan synthesis during remesothelialization was mediated through an increase in HAS II mRNA expression with a concomitant decrease in HAS III expression [8]. The ability of mesothelial cells to manifest features of mesenchyme underscores the plasticity property of these cells. The early phase of remesothelialization was dependent primarily on cell migration, and cell proliferation was observed during the latter part of mesothelial replenishment. Mesothelial cells reassumed their cobblestone, epithelial morphology once the monolayer was reestablished and cell-cell contact restored, and this was associated with a decrease in hyaluronan synthesis. The importance of hyaluronan in mesothelial regeneration was highlighted in separate studies, whereby the rate of remesothelialization increased with rising concentration of exogenous hyaluronan added to the denuded monolayer [8]. The significance of hyaluronan in reepithelialization and wound healing was corroborated in the skin and shown to depend on its interaction with its cell surface receptor CD44, since interference of hyaluronan-CD44 interaction resulted in delayed wound healing and defective skin elasticity [83].

5. The Effect of PD on Hyaluronan Synthesis and Mesothelial Cell Function

Acute inflammation is a defense mechanism that has evolved in response to tissue injury. It is characterized by increased vascular permeability, cell infiltration to sites of injury, release of inflammatory mediators by both resident and infiltrating cells, and increased matrix turnover. Although inflammation may initiate temporary tissue damage, subsequent reparative processes promote the resolution of inflammation. Repeated insult to the peritoneal membrane as observed in PD, however, disturbs the reparative process, which results in chronic inflammation and subsequent destruction of the tissue.

5.1. PD Increases Hyaluronan Synthesis in Mesothelial Cells. Hyaluronan is often used as a surrogate marker of inflammation. We and others have demonstrated that low levels of hyaluronan can be detected in dialysis effluent obtained from noninfected PD patients and that these levels are significantly increased in PD patients with peritonitis [16, 80]. Our observation that serum hyaluronan levels did not differ between noninfected and infected PD patients and that dialysate hyaluronan levels are almost 2- and 10-folds higher that the corresponding serum levels in noninfected and infected PD patients, respectively, would imply that local production accounts for the hyaluronan detected in PD fluid [16]. Biochemical analysis of hyaluronan purified from noninfected and infected dialysis effluent showed it to be of a HMW with a hydrodynamic size of 0.75 as determined by Sephacryl-S1000 gel filtration [16]. We have demonstrated that over 90% of hyaluronan synthesized by cultured mesothelial cells under basal conditions is secreted into their culture medium and its molecular weight is identical to that detected in PD fluid. This together with our observation that both noninfected and infected PD fluids can induce *de novo* synthesis of hyaluronan in mesothelial cells would indicate that mesothelial cells are a likely source of dialysate hyaluronan [16].

The molecular weight of hyaluronan retained on the surface of mesothelial cells is larger than that secreted suggesting partial depolymerization of the parent molecule as it is released from the plasma membrane [16]. Once released by mesothelial cells, hyaluronan appears stable and does not further undergo fragmentation in the peritoneal cavity despite ongoing peritoneal inflammation [16]. Increased synthesis of hyaluronan in mesothelial cells during peritonitis is attributed to pro-inflammatory cytokines and growth factors, in particular IL-1β [63, 80]. The inability to detect hyaluronan fragments in spent PD fluids corroborates previous reports that LMW hyaluronan is not observed *in situ* [84].

5.2. Altered Hyaluronan Content in the Mesothelial Glycocalyx during PD. Commencement of PD in patients with end-stage renal disease is associated with the dilution of the glycocalyx's constituents and their subsequent removal from the peritoneal cavity with each exchange of PD fluid. A loss of the protective glycocalyx surrounding mesothelial cells would imply increased susceptibility of these cells to the detrimental effect of PD fluids. Studies have demonstrated that disruption of the glycocalyx in human smooth muscle cells using hyaluronan oligosaccharides inhibited cell migration and proliferation and resulted in a marked change to their morphology with the appearance of a flattened, more adherence phenotype [51]. We and others have demonstrated that PD fluid or its constituents can induce senescence in cultured mesothelial cells and such cells can also be isolated from spent PD fluid [26, 85–88]. Mesothelial cells with a senescent

phenotype have reduced cell proliferation, are more adherent to their substrate, and have increased fibrogenic properties [87, 89, 90]. Furthermore, ultrastructural studies have highlighted a reduction in the number and length of microvilli on these senescent cells compared to normal mesothelial cells [90]. Since hyaluronan plays a crucial role in the formation of microvilli, the unphysiological concentration of glucose in PD solutions could exert its harmful effects through altered hyaluronan synthesis and/or increased release from the mesothelial glycocalyx into the peritoneal cavity, and such effects would result in phenotypic changes and altered cellular functions. The observed increase in hyaluronan levels in dialysis effluent may in part be attributed to its release from the mesothelial glycocalyx. In line with this suggestion, hyperglycemia has been shown to reduce the volume of the endothelial glycocalyx in diabetic patients, which was associated with increased release of glycocalyx constituents into the circulation and perturbed vascular functions [91].

The importance of hyaluronan in maintaining the structural and functional integrity of the peritoneum during PD is highlighted in rat models of PD in which hyaluronan supplementation significantly reduced peritoneal inflammation, preserved the structural integrity of the peritoneum, and improved ultrafiltration and membrane transport functions [92–96]. Furthermore, *in vitro* studies whereby peritoneal mesothelial cells were incubated with exogenous HMW hyaluronan resulted in reduced secretion of fibronectin and inflammatory mediators, thus confirming the anti-inflammatory and antifibrotic properties of hyaluronan [97]. Hyaluronan supplementation may replenish peritoneal levels of hyaluronan and decrease the hydraulic conductivity of the submesothelium, thereby preventing PD fluid absorption [95, 98].

5.3. PD Increases Submesothelial Expression of Hyaluronan. In a recent study, Osada et al. assessed the intraperitoneal expression of hyaluronan in PD patients and healthy controls [99]. These researchers observed a weak expression of hyaluronan in the peritoneum of healthy subjects, whilst increased expression of hyaluronan was noted in the submesothelium of uremic patients who had just entered the PD program. Submesothelial expression of hyaluronan was further increased in chronic PD patients and also in patients who had developed peritonitis [99]. Although Osada et al. did not investigate the mechanism that resulted in an increase in intraperitoneal expression of hyaluronan, it may be attributed to increased levels of proinflammatory and fibrotic cytokines in the peritoneal cavity [14, 100–102] since PDGF, IL-1β, IL-6, TNF-α, and TGF-β1 have all been shown to increase hyaluronan secretion in cultured mesothelial cells and fibroblasts [18, 63]. Overexpression of hyaluronan is indicative of a breakdown in the fine balance between synthesis and degradation. These researchers did not provide any details of hyaluronan expression in the mesothelium, the molecular weight of hyaluronan expressed within the submesothelium, nor HAS activity in the peritoneum, and we were unable to determine whether peritoneal specimens used in their study were partially or completely devoid of a mesothelium, a phenomenon frequently observed in PD patients [6]. Changes in the submesothelium has been shown to precede the loss of the mesothelium in PD patients and is associated with peritoneal fibrosis and vasculopathy [6]. Is it therefore possible for increased submesothelial expression of hyaluronan to induce mesothelial denudation and peritoneal fibrosis? In an attempt to answer this, one must first explore the mechanism that upholds the integrity of an epithelial monolayer and the consequences of altered cell phenotype and subsequent pathologic EMT, with particular focus on hyaluronan in these processes.

5.4. Putative Role of Hyaluronan in EMT and Peritoneal Fibrosis. The expression of E-cadherin is a cardinal feature of epithelial monolayers [103, 104]. A loss of E-cadherin at the intercellular junctions is strongly associated with epithelial dedifferentiation, phenotypic alterations, and induction of EMT. E-cadherin is a calcium-dependent transmembrane glycoprotein that is localized at the basolateral membrane in adherens junctions and endows epithelial cells with their apicobasolateral polarity [104, 105]. E-cadherin is a suppressor of CD44-hyaluronan interactions and has been shown to prevent epithelial dedifferentiation and subsequent EMT [106]. A balance between E-cadherin and CD44-hyaluronan is therefore essential to maintain normal epithelial cell integity and function. In the setting of PD, decreased expression of ZO-1, E-cadherin, and β-catenin is observed in cultured mesothelial cells upon stimulation with elevated glucose concentration [33]. A reduction in E-cadherin expression in mesothelial cells would indicate a loss of the cells' ability to suppress hyaluronan expression. We have previously demonstrated that increased hyaluronan expression is critical for inducing EMT in mesothelial cells during wound healing and tissue repair but, once the mesothelial monolayer is replenished, hyaluronan levels return to constitutive levels [8]. The persistent increase in intraperitoneal expression of hyaluronan during PD would suggest that mesothelial cells are sustained in an activated fibroblastic phenotype (pathologic EMT) and mesenchymal-to-epithelial transdifferentiation is inhibited. Increased production of hyaluronan and its accumulation in the ECM has been shown to diminish contact inhibition in nontransformed rat 3Y1 cells [52], thereby underscoring the importance of hyaluronan in physiologic and pathologic EMT. Under physiological conditions, epithelial cells will undergo apoptosis if they are detached from their substratum [107]. However, epithelial cells that have undergone EMT have developed specific mechanisms that allow them to survive as anchorage-independent cells without cell-cell interactions [53]. Emerging evidence suggests that hyaluronan triggers numerous signaling pathways that promote cell survival of transdifferentiated cells [108]. The acquisition of a migratory and invasive phenotype allows mesothelial cells to migrate into the submesothelium following the disruption of the basement membrane, which is made possible by the increased production and activity of MMPs [86]. Transdifferentiated mesothelial cells adopt a more fibrogenic characteristic that allows them to contribute to the thickening of the submesothelium and subsequent peritoneal fibrosis [109, 110]. The migration of mesothelial cells into

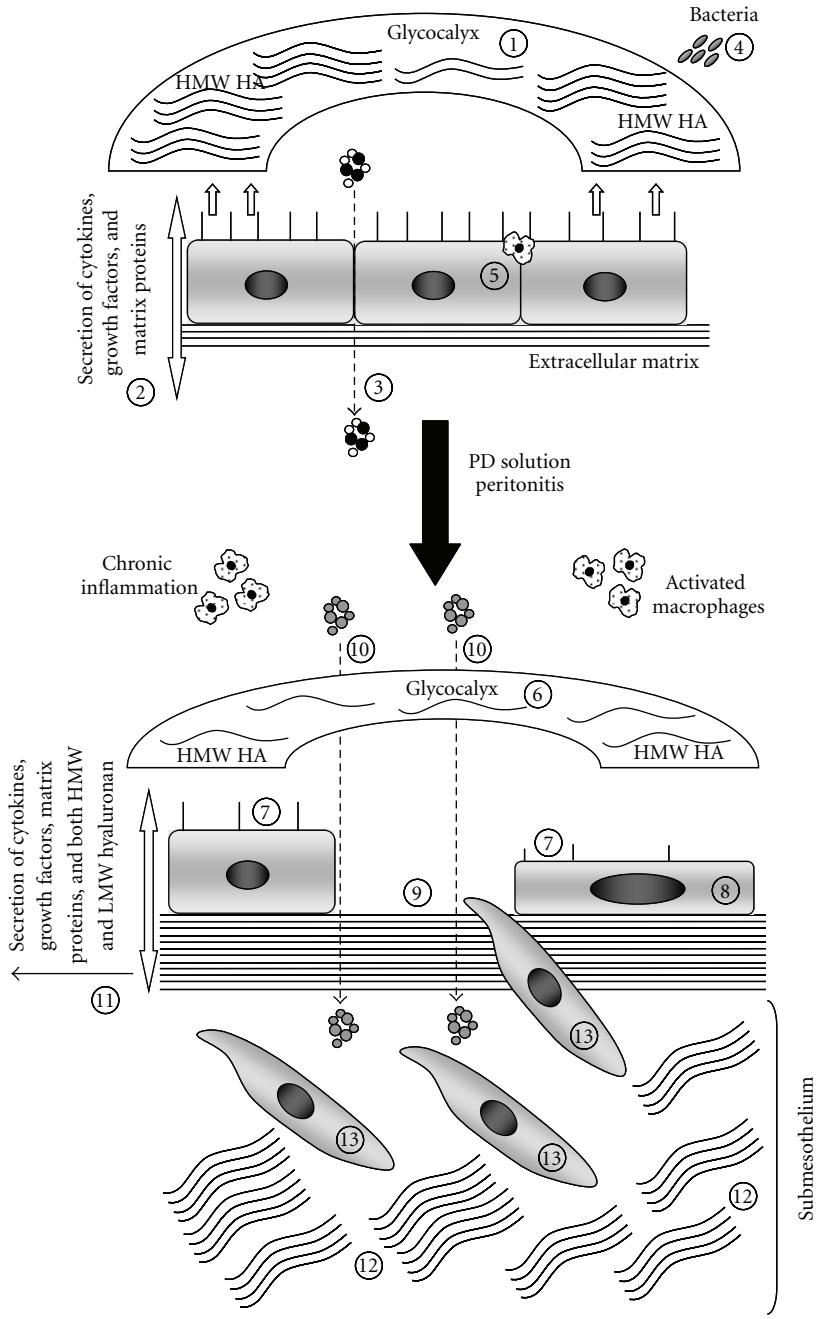

Figure 1: Changes to hyaluronan synthesis during PD alter mesothelial cell functions. The mesothelium contributes to peritoneal homeostasis. Under physiologic conditions, mesothelial cells secrete HMW hyaluronan which is the main constituent of the glycocalyx. The glycocalyx surrounds the apical surface of mesothelial cells and provides a protective barrier against abrasion, and a slippery, nonadhesion surface for intracoelomic movement (1). Mesothelial cells participate in tissue repair and in the induction and resolution of peritoneal inflammation through their ability to synthesize cytokines and growth factors that are secreted into the peritoneal cavity (2). Mesothelial cells also synthesize matrix proteins which provide a substratum onto which mesothelial cells adhere (2). Mesothelial cells facilitate the transport of fluids and solutes across the peritoneal membrane (3), are the first line of defense against bacterial peritonitis (4), and can maintain a chemotactic gradient to assist in leukocyte infiltration (5) during bacterial, chemical, or surgical insult. Changes to the peritoneum following the initiation of PD. Constant exposure of the peritoneal membrane to bioincompatible PD solution results in either a reduction of the glycocalyx volume (6) or its complete depletion from the surface of mesothelial cells. Changes in the content of hyaluronan in the peritoneum can induce morphologic and phenotypic changes to mesothelial cells that include reduced length and density of microvilli on the surface of mesothelial cells (7), generation of senescent cells (8), and frequent denudation of the mesothelium (9), thereby allowing PD solutions to leak into the submesothelium causing peritoneal injury (10). Activation of both immune cells and mesothelial cells further increases synthesis of cytokines and growth factors (11), which exacerbates peritoneal inflammation, fibrogenesis, and increases submesothelial expression of HMW hyaluronan (12). Activation of mesothelial cells induces EMT (13), breakdown of the basement membrane, and their migration into the submesothelium. Communication of activated mesothelial cells with peritoneal fibroblasts and endothelial cells may provoke further inflammatory and fibrotic processes in the submesothelium resulting ultimately in peritoneal fibrosis.

the submesothelium may also contribute to the denudation of the mesothelium, mediated in part through increased bioactivation of TGF-β1, a key mediator of tissue fibrosis [110]. An inability to restore the mesothelial monolayer is associated with unfavorable structural and functional changes in the peritoneal membrane of PD patients [6].

What is the functional role of hyaluronan in the submesothelium? Unfortunately, it is not possible to provide direct evidence of its contributing role to peritoneal fibrosis due to a lack of research in this area. Nevertheless, by extrapolating data from other studies, one may conceive that sustained increase in intraperitoneal expression of hyaluronan prolongs pathologic EMT and inflammation leading ultimately to peritoneal fibrosis. In this respect, high-molecular-weight hyaluronan has been demonstrated to induce Snail2 in NIH-3T3 mouse fibroblasts, which endow the cells with an increased invasive activity [111]. HMW hyaluronan has also been shown to upregulate MMP activity in lymphoma cell lines and metastatic tumor cells through the prior induction of NFκB [112]. Accumulation of hyaluronan in keratocytes mediates TGF-β1 induction of α-smooth muscle actin and matrix protein synthesis [62]. That hyaluronan can augment fibrotic processes is corroborated by the observation that gene silencing of HAS II or removal of hyaluronan by digestion with hyaluronidase inhibited TGF-β1-induced α-smooth muscle and fibronectin mRNA [62]. Taken together, these data would suggest that overexpression of hyaluronan plays a critical role in the induction of cell activation and tissue fibrosis, its transformation from a molecule that can exert a beneficial effect on tissue homeostasis to one that mediates tissue injury is dependent largely on its tissue concentration, localization, and molecular weight.

6. Hyaluronan as a Surrogate Marker for Peritoneal Inflammation

Deleterious changes to the structural integrity of the peritoneal membrane are often associated with a loss of dialytic potential in PD patients, which can lead to technique failure. Thickening of the submesothelium is a consistent observation in PD patients [6]. Studies have highlighted that the bioincompatible nature of PD solutions is a major instigator of EMT and peritoneal fibrosis. Although examination of peritoneal biopsies allows clinicians to monitor morphologic changes in the peritoneal membrane with time on PD, this method is invasive and cannot be performed frequently. The measurement of pro-/anti-inflammatory and fibrotic markers in dialysate effluent has provided researchers with a noninvasive method to indirectly assess the physical condition of the peritoneum with time. The measurement of hyaluronan in spent PD solutions provides researchers and clinicians with an indication of the degree of peritoneal inflammation with time and treatment regimens. In a quest to preserve the functional properties of the peritoneal membrane, new PD solutions have been developed that use amino acids or icodextrin as the osmotic agent instead of glucose or the partial substitution of lactate with bicarbonate [113–115]. The use of alternative PD fluids is associated with a reduction of proinflammatory mediators such as hyaluronan and a concomitant increase in CA125 levels, a marker of mesothelial cell mass and peritoneal membrane integrity [101, 102, 116, 117].

The levels of hyaluronan in biological fluids are a good indicator of numerous diseases and inflammation. Studies have demonstrated strong correlations between serum hyaluronan levels and the degree of impaired renal function [118, 119]. Increased serum levels of hyaluronan have also been shown to predict mortality and morbidity in PD patients [120], whereas dialysate levels of hyaluronan can predict survival in PD patients [121].

7. Conclusion

PD is an effective modality of renal replacement therapy for patients with end-stage renal disease, but, due to the bioincompatible nature of PD solutions, a chronic inflammatory response is generally observed within the peritoneal cavity, which jeopardizes the structural and functional integrity of the peritoneal membrane. Structural changes begin with the modification of the mesothelial monolayer, with a loss of microvilli, widening of the intracellular gap junctions, loss of E-cadherin, the adoption of a migratory, fibroblastic phenotype, and induction of EMT, which progress to peritoneal fibrosis. Studies have demonstrated that each of these processes is regulated or influenced by hyaluronan. It is intriguing that different molecular weights or concentrations of hyaluronan could result in distinct effects on biological processes. Pathologic changes in the peritoneum are attributed solely to HMW hyaluronan and not its fragments. Our knowledge of the contributing role of hyaluronan in peritoneal homeostasis and pathology, its interaction with its binding proteins, and how its synthesis is modulated during inflammation and fibrosis is far from complete. It is envisaged that, with time, we may unravel the many mysteries of hyaluronan, which will allow us to devise novel therapeutic interventions to preserve the structural and functional integrity of the peritoneum and thereby improving patient survival on PD.

Figure 1 shows a schematic diagram that details the current understanding of how hyaluronan protects the peritoneum during peritoneal homeostasis (upper panel) and how changes to its level and localization can mediate changes to mesothelial phenotype, function, and induce peritoneal injury in the setting of PD (lower panel).

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