Peritoneal Fibrosis—Can It Be Prevented or Slowed?

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No Disclosures
Take-Home Points

• After an insult, fibrosis occurs in all organs due to the formation and proliferation of myofibroblasts. Understanding the complex pathways may lead to methods of prevention of the process.

• Studies with cell lineage tracing demonstrate that epithelial de-differentiation along with fibroblast to myofibroblast transition account for repair mechanisms. Other theories include stem cell migration from the bone marrow, transformation of endothelial or mesothelial cell transition to myofibroblasts.

• Major insults to the peritoneum are the PD catheter (foreign body that forms biofilm), high glucose concentrations in dialysate, and overt bacterial infection.

• Needed: better biomaterials that preclude bacterial biofilm formation.

• Needed: improved solutions: Are there alternatives to glucose?

• Needed: development and clinical evaluation of pharmacologic measures to decrease inflammation and to preserve the peritoneum.
Topics

• **Fibrosis: How does it occur?**
• Causes of Peritoneal Fibrosis
• Potential Methods to decrease peritoneal fibrosis:
  – New catheter materials
  – New solutions
  – Potential pharmacologic additives to current dialysis solutions
Overview of Wound Repair and Fibrosis

Infections
Toxins
Drugs
Trauma
Recurrent inflammation

Epithelial/endothelial damage

Recurrent inflammation
TSLP
IL-25
IL-6
IL-33
TNF

Adaptive immune activation

Innate immune activation

Fibroblast activation

Fibroblast activation

Tissue repair

Myofibroblast activation

Inflammation and cell recruitment

↑ Smooth muscle actin
↑ Collagen synthesis
↑ Matrix deposition

Irritant removed?

YES

Healing and homeostasis

NO

Persistent irritant

Persistent myofibroblast activation

Fibrosis

Ab

IFN-γ

PMN

EOS

Macrophage

Baso

Mast

IL-18

TGFβ1

IL-17

↑ Smooth muscle actin
↑ Collagen synthesis
↑ Matrix deposition

There are multiple pathways and cells involved in fibrosis.

Another Part of the Puzzle

Role of Tyrosine Kinase Receptors in Peritoneal Fibrosis

Is peritoneal fibrosis like that of the kidney, liver, lung, and skin?
Lineage Tracing Reveals Distinctive Fates for Mesothelial Cells and Submesothelial Fibroblasts during Peritoneal Injury

JASN 25:2847-2858, 2014

Yi-Ting Chen,*†‡ Yu-Ting Chang,* Szu-Yu Pan,† Yu-Hsiang Chou,*† Fan-Chi Chang,*† Pei-Ying Yeh,* Yuan-Hung Liu,§ Wen-Chih Chiang,† Yung-Ming Chen,† Kwan-Dun Wu,† Tun-Jun Tsai,† Jeremy S. Duffield,| and Shuei-Liong Lin*† National Taiwan University

- Used inducible genetic fate mapping to trace cells after inflammation in 3 transgenic mouse (C57BL6) animal models (hypochlorite, hyperglycemic solutions, TGF-β1)
- Demonstrated mesothelial cells repair the mesothelium and myofibroblasts arise from submesothelial fibroblasts
- Found a possible target (PDGFRβ) for blockade to attenuate fibrosis
Model of Peritoneal Inflammation/Fibrosis

Chen YT et al  JASN 2014
In-vitro cultured mesothelial cells do not replicate in vivo results (Chen et al JASN 2014)

- Cultured primary mesothelial from WT1-RFP+ mice for ability to express αSMA in vitro
- No αSMA was seen with culture medium alone. With TGF-β1, these mesothelial cells expressed αSMA after 48 hours.
- However, there was no αSMA seen in vivo after over-expression of TGF-β1 by injection of Ad TGF-β1 in mesothelial cells in mice.
- This demonstrates the problems with planar cell culture models of a single type of cell.
More basic research is needed using lineage tracing and other molecular manipulations in order to sort out mechanisms and develop new targets for combating peritoneal fibrosis.
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• Fibrosis: How does it occur?
• **Causes of Fibrosis**
• Potential Methods to decrease peritoneal fibrosis:
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Introduction of Inflammatory Stimulus

Sub-Peritoneal Tissue Space

fibroblasts

hyaluronan collagen

endothelial cells

pericyte

interstitial matrix

muscle cells

basement membrane

Submesothelial Compact Zone

mesothelial cells

Catheter Glucose/GDP Bacteria

macrophage

peritoneal cavity
Mechanisms of Peritoneal Inflammation

Sub-Peritoneal Tissue

- fibroblasts
- MCPs, ILs, IL-6
- PGs, Rantes
- pericytes
- endothelial cell
- fibrinogen, fibronectin, albumin, IgGs, chemokines, PGs
- NOS
- vascular recruitment

Inflamed Submesothelial Compact Zone

- TGF-β1
- bFGF
- hyaluronan
- collagen
- interstitial matrix
- HA
- Col
- PD Solution (glucose, GDPs)

Peritoneal Cavity

- mesothelial cells
- PGs, Interleukins, hyaluronan, CA
- MCP-1, RANTES, CA125, VEGF
- IL-6
- IL-1, TNFα
- infectious agent
- macrophage
- H2O2
- catheter (biofilm)
PD Catheter = Foreign Body
Progressive inflammatory response over 1-7 days from sterile polyethylene catheters in rats

E. Gomez-Sanchez-Flessner
ASN 2010

Trichrome

control 1 day 3 days 7 days
Mouse Adherent Cell Layer at 2 weeks on Polyethylene Catheter
Peritoneal Inflammatory Response

Catheter Only

thickness (µm)

vessel number/mm

duration of exposure (weeks)

CON  4  8  20  20

low-GDP soln daily injection
Bacteria on Catheters in patients undergoing PD  Pihl M  PDI 2013;33:51

- Cultured cells from catheters of 15 patients without signs of infection
- Visualized bacteria with confocal microscopy
- Detected bacteria on 12/15 catheters
- Most common: Staph Epidermidis
- Up to 4 different species on some catheters

**These organisms exist in the catheter biofilm and likely provoke a continuous inflammatory state in asymptomatic patients, leading to subsequent peritonitis.**
Staphylococcal biofilm on peritoneal catheter  

Dasgupta Sem Dial 15:338, 2002
Introduction of Inflammatory Stimulus

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Submesothelial Compact Zone

mesothelial cells

Catheter Glucose/GDP Bacteria

macrophage

peritoneal cavity
Normal Plasma Glucose Concentration = \textbf{100 mg/dL}

Glucose concentrations in the dialysis solution:

\begin{align*}
1.5\% &= 15 \text{ g/L} = 1500 \text{ mg/dL} \\
2.5\% &= 25 \text{ g/L} = 2500 \text{ mg/dL} \\
4.25\% &= 42.5 \text{ g/L} = 4250 \text{ mg/dL}
\end{align*}
Peritoneal Cavity

Peritoneum

Blood Capillary

Mesothelium

Glycocalyx

Glucose-concentration osmolar profile

Glucose in PD fluid

Macrophage

Cell-extracellular matrix

Interstitium

Fibroblast

Pericyte

Muscle cells

Lymph vessel

0.6 - 1.0 mm
Davies et al. KI 67:1609, 2005

Entire Cohort

High Glucose

Low Glucose

No Icodextrin

Icodextrin

No peritonitis

1 or more episodes
Topics

• Fibrosis: How does it occur?
• Causes of Fibrosis
• Potential Methods to decrease peritoneal fibrosis:
  – New catheter materials
  – New solutions
  – Potential pharmacologic additives to current dialysis solutions
Can we decrease or eliminate inflammation induced by the catheter?
Can we decrease or eliminate bacterial biofilm?
Molecular Determinants of Biocompatibility


- Host cells interact predominantly with adsorbed plasma proteins, which have altered conformations and present sites for cell binding.
- Exposure of the P1/P2 epitopes of fibrinogen bound to biopolymers may be critical for binding of inflammatory cells.
- Different biopolymers have different affinities for protein-cell interactions.
- Blockade of protein sites alters cell binding.
Quantitative binding site measurement versus material

Phagocyte accumulation on various biomaterials.

PET = dacron; PE = polyethylene; PVC = polyvinyl chloride; PEU = polyurethane; PDMS = polydimethyl siloxane

Biocompatible?

Organs-on-a-Chip (large US-NIH Consortium that is entitled Microphysiological Systems):

Has demonstrated that cells grown directly on PDMS (polydimethyl siloxane) **had properties that were not consistent with in vivo conditions**.
Surface Modification of Silicone for Biomedical Applications Requiring Long-Term Antibacterial, Antifouling, and Hemocompatible Properties

- Covalently grafted poly(poly(ethylene glycol) dimethacrylate) – P(PEGDMA)) to medical silicone
- Added polysulfobetaine polymer (P(DMAPS)) to enhance anti-fouling
4 hr Exposure of polymers to S. aureus \(10^8 \text{cells/ml}\) Li et al; Langmuir 28:16408; 2012
3T3 Fibroblast adherence to polymers (24 hr) Li et al. Langmuir 28:16408; 2012
Platelet adherence to polymers (1 hour) Li et al. Langmuir 28:16408; 2012
Kocuran-functionalized silver glyconanoparticles as antibiofilm coatings
Kumar CG et al Nanotechnology 25:: 32510; 2014

- Synthesized silver 12 nm spherical glyconanoparticles (AgNPs) using Kocuran (exopolysaccharide produced by Kocuria rosea strain BS-1)
Silver nanoparticle based antibacterial coatings  
Taheri S et al  Biomaterials  35:4601; 2014

- Examined effect of low-amperage DC current exposure on established bacterial and fungal biofilms
- Time and dose (0-500 μA)-dependent bacterial killing observed with DC delivered via an intraluminal platinum electrode
- After 24 hours, 500 μA sterilized the catheter of all bacterial species tested

• Utilized 3-layers:
  – copolymer with zwitterion/quaternary ammonium side groups
  – Contact biocidal derivative of that polymer with octyl groups
  – Antibacterial hydrogen peroxide producing enzyme cellobiose dehydrogenase
  – Assembled these layer by layer

• Decreased bacterial adherence by 60%
Catheter Inflammation and Biofilm

- Remains a major problem for IP, IA, IV, and urethral applications
- Biomaterials are needed to decrease protein adsorption and cell adhesion
"Biocompatibility" and lower glucose are reasonable goals, but they have not proven 100% effective in animal experiments or in randomized human trials.

How about a new osmotic solute?

**Impact of low-glucose PD regimen on fibrosis and inflammation markers**  Yung S et al  PDI  35:147; 2015

Studied 43 patients on Physioneal, Extraneal, and Nutrineal (PEN group) vs Controls on Dianeal for 12 months

At 12 months: dialysate levels of CA125, decorin, HepGF, IL-6, adiponectin, adhesion molecules were significantly greater in PEN group compared to controls
Needed:
Alternative Osmotic Agent to Glucose

- Does not produce GDP or other harmful substances
- Does not produce metabolic changes
- Does not increase uremia
- Does not inflame or alter the peritoneal barrier
- Exerts effective osmotic pressure over 4-6 hours
- Is relatively inexpensive
HYPERBRANCHED POLYGLYCEROL IS AN EFFICACIOUS AND BIOCOMPATIBLE NOVEL OSMOTIC AGENT IN A RODENT MODEL OF PERITONEAL DIALYSIS


Tested HPG, 3kDa polymer in 2.5-15% concentrations (osmolality: 279-424 mOsm/kg) in same electrolyte solution as Dextrose solution.

Compared UF, urea clearance to 2.5% Dextrose: both were superior at 7.5-15% to the 2.5% D solution.

Histology of abdominal wall and WBC flow cytometry demonstrated better biocompatibility.
HPG: Questions/Concerns prior to Human Testing

- Metabolism of hyperbranched polyglycerol?
- Use in uremic animals?
- Multiple exchanges: efficacy and biocompatibility?
- Toxicity? Deposition in cells/organs?
- Rodents vs humans?
Topics

• Fibrosis: How does it occur?
• Causes of Fibrosis
• **Potential Methods to decrease peritoneal fibrosis:**
  – New catheter materials
  – New solutions
  – **Potential pharmacologic additives to current dialysis solutions**
New catheter materials and a new osmotic agent in PD are desirable but require years of development and safety testing.

Can we use additives to current solutions to decrease inflammation and damage to the peritoneum?
## Pharmacologic Targets and Peritoneal Remodeling

(Farhat et al PDI 2014; 34:114)

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Novel Additives to Current PD Solutions

- **Decorin** (proteoglycan inactivates TGF-β) administered via gold nanoparticles or adenovirus decreases peritoneal fibrosis in an in vivo rodent model. Chaudhary K AJP 307:F777; 2014
Additives (cont’d)

- **Suramin** inhibits the development and progression of chlorhexidine-induced peritoneal fibrosis.  
  Xiong C et al JPET 351:373; 2014
Additives (cont’d)

Additives (cont’d)

- **MicroRNA-29b** inhibits peritoneal fibrosis in mouse model of PD  
  Yu JW et al Lab Invest 94:978;2014

UT = untreated  
EV = empty vector  
miR-29b-vector
Additives (cont’d)

- Janus kinase signaling activation mediates peritoneal inflammation; Effect of **JAK 1/2 inhibitor** after 10 days of 10 ml BID PD in rats  
  
  Dai T Kidney Intl 2014

Saline dialysate  4.25% Dianeal  4.25% Dianeal + JAK 1/2 Inhibitor

Abdominal wall

viscera
Oral Paricalcitol decreases Peritoneal Remodeling during PD


Control  5 wks – PD Fluid  5 wks- PDF +Paricacitol
Inhibition of EGF Receptor Blocks Development and Progression of Peritoneal Fibrosis  
Wang I et al  JASN 2016

• Examined use of gefitinib, a specific inhibitor of EGFR, on development/progression of peritoneal fibrosis in rats daily-injected with chlorhexidine gluconate

• Administration of gefitinib directly after chlorhexidine injection prevented onset of fibrosis

• Delayed administration of gefitinib after fibrosis onset halted fibrosis progression and abrogated increased: phosphorylation of EGFR, Smad3, NF-κB, and TGF-β1

• Reduced angiogenesis in the tissue

• **Results demonstrate importance of EGFR in peritoneal fibrosis, inflammation and angiogenesis**
Human Umbilical Mesenchymal Stem Cells Treat Rat Peritoneal Dialysis Induced Fibrosis

Additives (cont’d)

• **Endothelin-1** may be a target for prevention of peritoneal dialysis-associated fibrosis. Busnadiego O et al. JASN 26; 2014.

• **SMAD2 and SMAD3** play opposing roles in peritoneal fibrosis and are potential targets. Duan W-J et al. Am J Pathol 184:2275; 2014.

• Oral **Astaxanthin** supplementation prevents peritoneal fibrosis in rats. Wakabayashi K et al. PDI 2013.

Additives (cont’d)

• **Telmisartan** attenuates peritoneal fibrosis via peroxisome proliferator-activated receptor-γ activation in uremic rats  
  Su X et al  

• The **Kampo Medicine Daikenchuto** inhibits peritoneal fibrosis (induced with chlorhexidine) in mice by inhibiting inflammation and HSP47 expression.  
  Kitamura M et al  
  Biol Pharm Bull  38:193; 2015
While these pre-clinical studies are promising, each of these agents require clinical confirmation in government qualified trials.
Take-Home Points

- After an insult, fibrosis occurs in all organs due to the formation and proliferation of myofibroblasts. Understanding the complex pathways may lead to methods of prevention of the process.
- Studies with cell lineage tracing demonstrate that epithelial de-differentiation along with fibroblast to myofibroblast transition account for repair mechanisms. Other theories include stem cell migration from the bone marrow, transformation of endothelial or mesothelial cell transition to myofibroblasts.
- Major insults to the peritoneum are the PD catheter (foreign body that forms biofilm), high glucose concentrations in dialysate, and overt bacterial infection.
- Needed: better biomaterials that preclude bacterial biofilm formation.
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Thank you for your attention!

Questions?