Analysis of Primary Urethral Wound Healing in the Rat

Matthias D. Hofer, Earl Y. Cheng, Matthey I. Bury, Eugene Park, Wei Xu, Seok Jong Hong, William E. Kaplan, and Arun K. Sharma

OBJECTIVE To analyze the process of urethral healing, which is the basis of urethral reconstructive surgery but remains poorly understood, we have developed a rat model of urethroplasty. Understanding this process may provide strategies to prevent aberrant urethral healing and improve the healing process.

METHODS We performed urethroplasties on 36 male Sprague-Dawley rats. On postoperative days 2, 4, 6, 8, 10, and 12, animals were sacrificed. The number of neutrophils, macrophages, fibroblasts, blood vessels, and Ki67 proliferative index was evaluated with immunostaining and collagen I and III contents with picrosirius staining. Expression of *VEGF*, *PDGF*, *TNFα*, *TGFβ*, and *FGF* was analyzed with quantitative real-time PCR.

RESULTS Urethral healing occurs in phases of inflammation, proliferation, maturation, and remodeling analogous to dermal healing, however, with extended duration of each phase. The inflammatory phase reached to postoperative day 4 being characterized by neutrophil and macrophage predominance and high levels of VEGF, PDGF, *TGFβ*, *TNFα*, and *IL-10*. The proliferative phase extended until day 10 characterized by myofibroblast proliferation and angiogenesis. Maturation and remodeling started on day 10 with decreasing proliferation and angiogenesis, increasing collagen I formation, and periurethral alignment of connective tissue. The healing process involved >50% of the periurethral/spongiosum area in the inflammatory and >80% in the maturation and remodeling phase.

CONCLUSION Urethral healing occurs in phases similar to those observed in dermal healing, however, with extension of each phase. The healing process is not limited to the site of injury but involves the vast majority of periurethral tissue and corpus spongiosum. This appears to be the result of the unique anatomical features of the urethra. UROLOGY 84: 246.e1–246.e7, 2014. © 2014 Elsevier Inc.

Surgical interventions involving the urethra, such as urethrotomy, hypospadias repair or urethroplasties, rely on functional wound healing to be successful. Complications of impaired wound healing include fistula development in which a sufficient scar fails to form, and urethral strictures as consequence of excessive fibrosis and cicatrix formation.[1] Similarly, traumatic events to the urethra of other etiology can also lead to urethral strictures subsequent to excessive scar formation during the healing process. However, despite the consequences of impaired wound healing, research in the field of urethral healing has been limited and knowledge of the urethral healing processes is rather sparse.

The urethra is histologically a unique organ with a specialized mucosa, underlying spongiosal tissue with large cavernous vascular spaces, Littré glands, and smooth muscle fibers, and encircled by fascia. Conceptually, urethral wound healing may resemble dermal wound healing and recapitulate the phases thereof.[2,3] Dermal healing, schematically depicted in Figure 1A, starts with the hemostasis phase, in which thrombocyte aggregation occurs, a fibrin clot is formed, and wound hemostasis achieved. In the following inflammatory phase characterized by neutrophil and macrophage invasion, tissue debris is removed and cytokines are released initiating the proliferation and migration of cells. The resulting proliferative phase is characterized by myofibroblast proliferation, angiogenesis, and production of collagen, mainly collagen III, when a provisional extracellular matrix is formed. In this phase, reepithelialization also occurs. Differentiation of fibroblasts into myofibroblasts results in wound contraction. In the maturation and remodeling phase, the provisional extracellular matrix is rearranged

Financial Disclosure: *The authors declare that they have no relevant financial interests.*
Funding Support: *This study was funded by the Ann and Robert H. Lurie Children's Hospital of Chicago Faculty Practice Plan.*

From the Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, IL; the Division of Pediatric Urology, Department of Surgery, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; the Department of Plastic Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL; the Institute for BioNanotechnology in Medicine (IBNAM), Northwestern University, Chicago, IL; and the Department of Biomedical Engineering, Northwestern University, Evanston, IL

Reprint requests: Matthias D. Hofer, M.D., Ph.D., Department of Urology, Northwestern University Feinberg School of Medicine, Tarry 16-703, 303 E. Chicago Avenue, Chicago, IL, 60611. E-mail: m-hofer@northwestern.edu

Submitted: March 13, 2014, accepted (with revisions): April 8, 2014

0090-4295/14/$36.00
http://dx.doi.org/10.1016/j.urology.2014.04.012

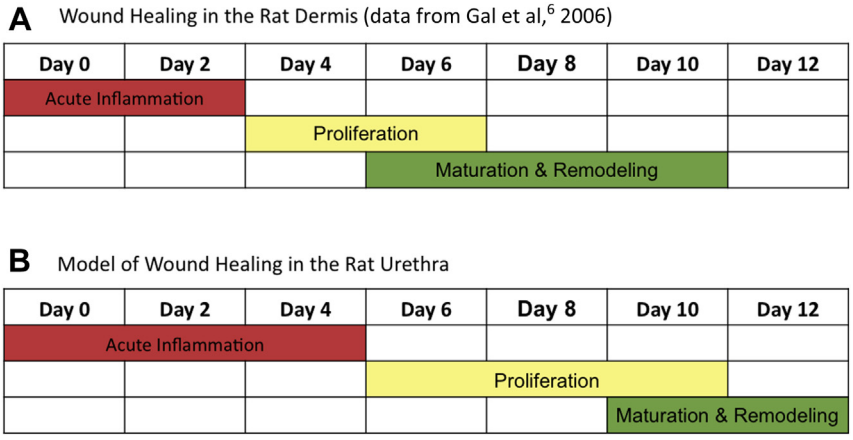

Figure 1. (A) Schematic depiction of the process of dermal healing and **(B)** urethral healing in the rat. Urethral healing recapitulates the phases of dermal healing, however, with extension of each phase.

and collagen III replaced by collagen I. Fibers are rearranged along tension lines, and the tensile strength of the scar consecutively increases.[2,3]

Similar phases of healing have been described for other mucosal surfaces, such as the oral cavity, and important differences compared with dermal healing have been found, for example, a far less prominent inflammatory phase.[4,5] On the other hand, findings from other mucosal surfaces cannot be simply extrapolated as they differ markedly. For example, scar formation is virtually absent during oral wound healing[5] but occurs in the urethra leading to stricture disease.

The aim of the present study was to analyze urethral healing in a rat model of urethroplasty and to describe the cascade of events after a surgical trauma to provide insight into the processes involved. This may allow for a better understanding of urethral healing and provide insights into how to ultimately enhance urethral regeneration and to avoid impairment of the healing process thus decreasing stricture and fistula rates. It may also provide the opportunity to develop interventions, for example, pharmacologic, which may aid in this task.

METHODS

Animals

Rats of ~300 g were obtained from Charles River Laboratories (Wilmington, MA). Animals were anesthetized with isoflurane (2% in oxygen). A 22G venipuncture catheter (Becton Dickinson, Franklin Lakes, NJ) was advanced into the urethra and secured in the glans with 7-0 Vicryl. Magnification provided by surgical loupes was sufficient for performing this procedure. A tourniquet around the base of the penis was applied, a circumcision incision made, and the penis degloved. A 0.8-cm vertical midline incision was made into the ventral urethra with a microknife and the venipuncture catheter exposed. The urethra and overlying corpus spongiosum were closed with running 7-0 polydioxanone suture (PDS). The tourniquet was removed (penile ischemia time <20 minutes to limit potential ischemic damage) and the venipuncture catheter retracted to the glans followed by retrograde injection of saline to check for leaks. If necessary, additional stitches with 7-0 PDS were applied to close the leak. The circumcision incision was closed with 7-0 PDS and Bacitracin applied. The animals received subcutaneous buprenorphine for postoperative pain control, which was continued as daily injection for 2 postoperative days. Animals were followed daily for distress caused by pain (posturing, vocalization when touched, wound infections, and dietary habits) or retention (additional abdominal distension and lack of voiding determined as dry bedding). The procedure from incision to closure of the circumcision incision took approximately 30 minutes per rat. On each postoperative day 2, 4, 6, 8, 10, and 12, six animals were sacrificed and the penis harvested (the operations were performed on 6 rats per day so that all animals of each operative day reflected the entire cohort to be harvested on a designated postoperative day). In total, 43 rats were used, 36 for the study and 7 to replace animals that did not survive until the scheduled day of sacrifice.

Tissue Preparation

Formalin-fixed tissue was gradually dehydrated and embedded in paraffin before generating 5-μm sections using a microtome. Hematoxylin-eosin staining was carried out using standard protocols.

For ribonucleic acid (RNA) extraction, the whole penis was snap frozen, embedded in Tissue-Tek optimal cutting temperature (Sakura Finetek, Torrance, CA) and sectioned with a cryostat and stored at −80°C. Integrity of tissue was checked with hematoxylin-eosin sections.

Immunohistochemistry

Slides were incubated at 61°C for 2 hours before antigen retrieval in a steamer by boiling for 20 minutes in antigen-retrieval solution with a pH of 6.1 (DAKO, Carpinteria, CA). Slides were washed in phosphate-buffered saline and antigens blocked with 1% bovine serum albumin (BSA). Primary antibodies for myeloperoxidase (goat anti-rat; Abcam, Cambridge, MA) and CD68 (rabbit anti-rat; Abcam, Cambridge, MA) were applied for 1 hour at a 1:100 dilution in 1% BSA, smooth muscle antigen (SMA, mouse anti-rat; Abcam, Cambridge, MA) was diluted 1:200 in 1% BSA. After three 5-minute washes in 1% BSA, the secondary antibodies, either Cy3 or Cy5 coupled, were applied in a 1:1000 solution in 1% BSA for 30 minutes. Slides were washed 3 times for 5 minutes with 1% BSA and coverslipped with 4′,6-diamidino-2-phenylindole