Neo-regeneration of urinary bladder: A desired metaplasia of autologous membrane from rectosigmoid colon containing stem cells of intestinal crypts

B G Matapurkar
Department of Surgery, Lok Nayak Hospital and Maulana Azad Medical College, New Delhi 110 001, India
A Bhargave
Senior Chief Medical Officer, Safdarjung Hospital, New Delhi 110 029, India.
H S Rehan
Department of Pharmacology, Lady Harding Medical College, New Delhi 110 001, India
A K Mondal
Department of Pathology, Maulana Azad Medical College, New Delhi 110 001, India
V K Ramteke
Department of Surgery, Maulana Azad Medical College, New Delhi 110 001, India.

Received 16 October 2006; revised 12 April 2010

The current management of diseases of urinary bladder requiring resection is by augmentation cystoplasty or transplantation of ureters. Transplantation of ureters is associated with morbidity and mortality. Ideal management will be by regenerating urinary bladder in vivo. Neo-regeneration of tissues and organs like abdominal wall, aponeurosis etc., has been attempted and patented. After neo-regeneration of mesoderm tissues and organs, regeneration of urinary bladder (developed from endoderm) was. In vivo surgical techniques were developed in dogs. It is known that the embryonic morphogenesis of urinary bladder is from urogenital sinus of hind gut. A membrane, containing endoderm stem cells in crypts of recto-sigmoid colon, was surgically isolated and colonized with remnant of urinary bladder wall after extensive resection. Experimental study was performed in dogs, for 60 days to one and a half year. Regeneration of all the layers of tissues of the wall of urinary bladder was observed. The neo-regeneration phenomenon has been recognized as “desired metaplasia”. The regenerated neo tissue/organ on histological examination and cystometry studies was found compatible with normal urinary bladder. The hypothesis, neo-regeneration and desired metaplasia, is discussed

Keywords: Adult stem cells, Desired metaplasia, Endoderm, Intestinal crypts, Neo-organo-histo- genesis, Urinary Bladder

Despite numerous attempts performed at augmentation cystoplasty by the use of biodegradable materials to induce regeneration of urinary bladder with varied success rates, its clinical use has not been possible due to associated complications of the modalities like infection, ulceration, calcification, stone formation and graft shrinkage. Morphogenesis of organs and tissues is by cellular proliferation and differentiation of a single celled fertilized ovum. Plants and lower life forms in nature have the capacity to regenerate lost or damaged body parts. Newts, salamanders, lizards, frogs etc. have capacity to regrow their lost organs. In higher animals, mammals and primates this capacity is rarely seen or absent. However, neo-regeneration of tissues and organs in mammals and primates is possible. An in vivo method of neo-organogenesis of different tissues and organs has been attempted, using autologous adult tissue stem cells in mammalian body. The adult stem cells are derived from the respective germ layers of a developing embryo. These are lineage committed progenitor cells differentiated from the totipotent embryonic stem cells. These adult stem cells have restricted potency limited to the germ layer to which they belong. This is evidenced from the metaplastic tissue forming capacity of these cells, under abnormal environmental conditions. Based on the proposed hypothesis on the principles of embryology, surgical techniques are developed. The technique comprises of surgically transferring of appropriate, autologous stem cells from adult tissue for colonizing the host tissue.
system where organ regeneration/repair is required. Selection of appropriate tissue cells is based on the hypothesis published elsewhere. The intestinal crypts have stem cells at their bases. These cells proliferate differentiate and at the same time migrate towards surface to replace intestinal cells, damaged during normal wear and tear. Present research relates to a method of isolating and developing an autologous membrane from recto-sigmoid colon with bases of colonic crypts containing pluripotent stem cells of endodermal origin. This membrane was used for the regeneration of urinary bladder as both the tissues are of endodermal origin and have developed in contiguous regions of the developing embryo. This communication critically analyses the neo-regeneration of urinary bladder, phenomenon of desired metaplasia, the supporting hypothesis and neo-regeneration.

Materials and Methods

After prior permission of Institutional Research and Ethical Committee, a prospective study was conducted on 7 healthy female Mongrel dogs of 3–4 years of age, weighing about 10 kg body weight, quarantined for 2-3 weeks duration.

All dogs were screened during quarantine period for routine blood and urine examinations. Urine and stool culture investigations carried out to rule out any infection.

Pre-operative preparation—Bowels were prepared with oral administration of metronidazole, (10 mg/kg body weight) for 5 days prior to surgery. Mechanical cleaning of intestine was performed with saline enema for three consecutive days. The dogs were fasted over night before surgery. In addition the recto-sigmoid bowel sterilized with administration of metronidazole retention enema, on the morning of operation. Cystometry was performed in all dogs preoperatively as control study.

Anesthesia—Intravenous pentobarbitone sodium (40 mg/kg body weight) was used to induce general anaesthesia. Intravenous line for dextrose-saline was established. The dogs were catheterized with self retaining, indwelling Foley’s catheter (5 F size) and connected to a draining receptacle under the table.

Experiment—Under peri-operative antibiotic cover and proper aseptic and antiseptic precautions, the abdomen was opened by infra-mbliical, supra-ubic midline incision. Recto-sigmoid colon was identified and isolated. Two to three inches of colon resection was carried out and kept in saline solution. The bowel continuity was established by usual standard surgical technique of colon-rectal anastomosis. The peritoneum was closed leaving the abdominal wall open for bladder exploration.

Preparation of stem cell membrane—The segment of colon obtained after resection was cleaned thoroughly with copious amount of saline water and en-sleeved on a conical centrifuge tube to facilitate further dissection of the segment (Fig. 1a). The outer layers of colon segment the serosa and muscularis externa were teased out and removed with blunt and sharp dissection. The colon tube was inverted inside out to expose the inner surface of the intestinal segment. Inner lining of epithelium and part of sub-mucous layer were also teased and dissected. Utmost care was taken not to puncture the remaining membrane. All free tags of dissected tissue (dead/devitalized tissue tags) on the cylinder of membrane were carefully removed from both sides of the cylinder. After dissection, the remaining colon was a tough cylinder of membrane. This cylinder consisted of thin layer of muscularis mucosa and part of sub-mucous layer containing bases of the crypts of the colon (Fig. 1b). During dissection utmost care was taken not to puncture or perforate the membranous cylinder. This is to avoid urine leak in post-operative period. The colon cylinder was slit longitudinally at the circumference to convert it into a flat membrane (Fig. 1c). The inner epithelial surface of the cylinder was carefully recognized and remembered as this surface is to be kept towards the lumen of urinary bladder while grafting it with the bladder remnant.

The presence of crypts was confirmed on microscopic examination of frozen section of the membrane (Fig. 1d). Thus prepared and obtained membrane was 1 mm thick and showed following layers:

- part of sub-mucous layer with loose areolar tissue containing blood vessels, lymphatic vessels, nerves etc.
• thin layer of muscularis mucosa.; and
• epithelium in immediate vicinity of muscularis mucosa containing cellular elements.

The multiple circular and half-moon like structures and cell groups were visible under the microscope. If these cells were hydrolyzed, traumatized or have lost configuration, the membrane was considered unsuitable for neo-regeneration/repair and was discarded. This is to ensure the functional viability of the cells.

Preparation of urinary bladder for colonization of stem cell membrane—Under antiseptic and aseptic precautions the abdomen was draped again and the urinary bladder exposed in extra-peritoneal space. The bladder capacity measured with 50 ml saline through the already inserted indwelling catheter. The urinary bladder wall was excised leaving a small rim of the wall around the trigone having ureteric and urethral openings. The rim was kept sufficiently wide to enable the grafting of the membrane. The prepared stem cell membrane from recto-sigmoid colon was now sutured to the remnant of the bladder with leak proof anastomosis (Fig. 2a). The epithelial surface of the membrane was carefully recognized and remembered to keep it towards the lumen of the urinary bladder. The capacity of the bladder was measured again and noted for comparison in subsequent exploration at the same time to confirm the leak proof anastomosis. Marker sutures (4/0 prolene suture) were applied over normal bladder wall all around the anastomosis. (Fig. 2b). The distance between markers was noted at planes right angle to each other for comparison with post operative state of the markers after 60 days onwards. The abdomen was closed in layers by usual surgical technique.

Post-operative management—The dogs were fasted for 24 h post operatively and nursed on intravenous fluids. All dogs were reexamined for anemia and repeated urine cultures for presence of urinary infection. The antibiotic cover continued for 24 h only, except in one dog the antibiotics continued for 5 days. Indwelling catheter was removed after the dog recovered from anesthesia (6 to 10 h post operative period). Liquid diet was started after 24 h. The stitches were removed after 8–10 days post-operative period.

Re-exploration—The dogs were re-operated after post-operative period of observation. Two dogs were observed for 60 days, 1 dog each for 90 and 180 days.

Fig. 1—Technique of isolation of endoderm stem cell membrane from hind gut. (a) isolated hind gut segment (recto-sigmoid colon); (b) thin cylinder of membrane developed by carefully dissecting outer layers of serosa, muscularis externa and inner layers of epithelium along with part of submucosa from the isolated segment of colon; (c) the cylinder converted into a flat membrane by slitting at the circumference of the cylinder; (d) histology of the isolated membrane (H & E; 10x10 magnification) Bases of the crypts of intestine containing stem cells near the tissue layer of muscularis mucosa (arrow).
2 dogs for 365 days and 1 dog for 545 days. The presence of ulceration or diverticulum formation was carefully noted. Record was also made for change in colour, texture, thickness and size of the membrane. Adhesions around urinary bladder were noted and recorded. The recto-sigmoid membrane along with urinary bladder was excised for gross and histological examination at the end of observation period. At the end of experiment, the dogs were euthanized, by giving high doses of pentobarbitone sodium. Serial sections of the grafted membrane were obtained and stained with Hematoxylin – Eosine (H & E) stain and with Masson’s Tri Chrome Stain (MTS) as per usual pathological laboratory techniques, for histopathological examination.

Cystometry—The study conducted under light sedation (intra venous pentobarbitone sodium, 10 mg/kg body weight). Urodynamic study conducted by using three lumen Foly's catheter (5 F size). The main port of the catheter was used for urinary bladder filling with saline, 2nd port for detrusor pressure recording and 3rd port for urethral pressure recording. The study was carried out in all the dogs (Table 1). The study included preoperative (control group) and in same dog in post-operative period.

Results
The post operative period was uneventful in all the dogs except in one dog which developed fever due to local wound infection which subsided after antibiotic treatment. The antibiotic cover was continued for 5 days. On repeated urine culture examinations, no urinary infection was observed in any dog. Initially urine voiding was uncontrolled and leaked through urethra. The urine voiding gradually improved to normal pattern. Later on, all the dogs voided urine normally after 60 days of operation. On re-exploration after observation period, the urinary bladder region showed few flimsy adhesions. These could easily be dissected by blunt dissection with gauze wrapped on finger. The adhesions were progressively stronger and thicker in later post-operative periods after 90 days but were easily dissectible without any damage to the organ. No ulceration or any diverticulum formation was observed on the region of the grafted membrane or around the bladder. No calcification of wall or stone.

Fig. 2—Regeneration of urinary bladder: Colonization of membrane containing stem cells. (a) Stem cell membrane colonized with urinary bladder tissue system after excising the bladder wall (note colour of the membrane), (b) membrane after 3 months post operative period (note changed colour of the membrane). (c) histology - 60 days post operative period. Transitional epithelium completely regenerated with sparse smooth muscle regeneration. Muscle regeneration is scanty (compare with fig. d). (d) histology - (D-1) Low Power (H & E; 10×10 magnification) 90 days post operative period. Regenerated serosa with near normal regenerated smooth muscle layer. (D-2) High power (H and E; 10×40 magnification)
formation was seen inside the urinary bladder in any of the dog in post operative period up to one and half year. No scarring of grafted membrane was observed in any dog. The urinary bladder distended normally after filling 50 ml of saline. There was no urinary leak in any of the operated dogs. The dimension of the bladder was measured between the markers at two different planes right angle to each other and was found to be unaltered. It measured almost same as on first exploration when sutures were applied, suggesting no shortening or contraction of the colonized membrane. The colour of the graft changed to dark red from pale white colour of the membrane (Figs. 2a and b). The demarcation over anastomotic line between normal bladder wall remnant and the grafted membrane could not be seen as both areas blended so well that it was difficult to differentiate the two surfaces on gross appearance (Fig. 2b). No scar could be noted at the junction line of normal and grafted membrane.

**Histology of the stem cell membrane post-operative**

**Gross examination**—The surface of the graft was red, smooth and glistening. Scar formation was not observed at the junction of bladder and graft membrane. The outer surface of membrane and normal bladder region was undistinguishable. Overall thickness of the grafted membrane at 60 days post-operative period, was not uniform all over the graft. It measured between 1 mm at places and up to 4 mm at other places. But later on, after 90 days post-operative period thickness was uniform all over the grafted membrane. From 90 days onwards the excised bladder was uniformly thick between 5.5 to 6.5 mm in different animals (Table 2). There was no devitalized area, ulceration, calcification, scar formation visible or diverticulum formation in the wall of the excised urinary bladder.

**Low power microscopy**: *(H & E stain 10x10 magnification)*—All the layers of urinary bladder wall, except outer layer of serous covering were present at 60 days post-operative sections. The serous layer was patchy and incomplete. The tissue sections after 60 days observation period, from colonized cell membrane region, showed sparse smooth muscle regeneration at different parts of the graft. The smooth muscle formation was seen not only at the edges but also simultaneously all over the colonized membrane sections. In later tissue sections at 90 to 180 days post-operative period the muscle regeneration was increasingly observed. The MTS stain showed uniform growth of smooth muscle by eosine stain. Complete covering of serous layer was observed after 90 days post-operative period. The microscopic sections after 90 days onwards, showed smooth muscle layer uniformly developed in distribution as well as in thickness in comparison to sections from normal urinary bladder remnant in the same animal (Fig. 2 C and D).

<table>
<thead>
<tr>
<th>Number of dogs</th>
<th>Bladder volume (ml)</th>
<th>Voiding pressure (mm of water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>52</td>
<td>At 60 days</td>
</tr>
<tr>
<td>55</td>
<td>At 60 days</td>
<td>not done</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>At 90 days</td>
</tr>
<tr>
<td>52</td>
<td>58</td>
<td>At 90 days</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>At 180 days</td>
</tr>
<tr>
<td>54</td>
<td>55</td>
<td>At 180 days</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>At 365 days</td>
</tr>
<tr>
<td>50</td>
<td>52</td>
<td>At 365 days</td>
</tr>
<tr>
<td>52</td>
<td>55</td>
<td>At 365 days</td>
</tr>
<tr>
<td>50</td>
<td>56</td>
<td>At 545 days</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>60 days post-operative period</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>60 not done</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>58 not done</td>
</tr>
</tbody>
</table>

*The bladder volume and pressure as compared to control group found to be grossly unchanged. As per the animal ethical guidelines number of dogs studied was too small for calculation of mean±SE.

<table>
<thead>
<tr>
<th>Dogs number</th>
<th>Urinary bladder wall thickness (mm) (Pre-operative)</th>
<th>Grafted membrane. Thickness after observation period after (Post-operative in the same animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>60 days post-operative period: 6 mm.</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>—do—</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>90 days post operative period: 6 mm.</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>180 days post operative period: 6 mm.</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>365 days post operative period: 6 mm.</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>5.5 mm.</td>
</tr>
<tr>
<td>7</td>
<td>6.5</td>
<td>18 months postoperative period: 6 mm.</td>
</tr>
</tbody>
</table>
High power microscopy: (H & E stain 10x40 magnification)—High power microscopic examination confirmed the findings as above. Regeneration of all the layers of urinary bladder wall observed in the cell membrane graft in tissue sections of 90 days and thereafter.

Masson’s Tri-chrom (M T S) stain—In H & E stain examination both fibrous and muscle tissues were stained by eosin stain. It is impossible to differentiate the two tissues. On MTS stain the fibrous tissue was stained green and muscle was stained by eosine. The colour differentiates the muscle tissue from fibrous tissue (Fig. 3). Interestingly, histological examination at 60 days post-operative period showed bundles of pale coloured tissues. These could not be stained by eosine or green in these sections. These unstained tissue bundles were not seen in examinations 90 days and after (vide discussion).

Urodynamic studies (Table 1)—The cystometry was carried out in all the dogs but data of five dogs were compared, as the study in two dogs of 60 days post-operative period was not performed and was not available (Table 1). The bladder volume of control studies ranged from 50–55 ml while it ranged 50–58 ml in post-operative study. Voiding pressure in control group ranged from 52–60 mm of water while in post-operative group it ranged from 55–59 mm of water.

Discussion

Despite the discovery of cell as the micro-unit of bodies of multicellular organisms, the cell only produce cells, and the knowledge that a single celled fertilized egg forms the entire body which comprises different tissues and organs, having different structure and function, the regeneration of abdominal wall aponeurosis could not be published by using adult tissue stem cells until 1991. The fact that a variety of blood cells in blood are produced from a few cells of the bone marrow came to be known only in 1917. A reasonable definition on stem cell could be possible after 135 years. Classically, even now the functional definition of tissue stem cell is the base line. Review of literature on the subject of regeneration of smooth muscle is very interesting. Standard bio-medical teaching in first half of the 20th century, believed that smooth muscles have very little or no regenerative capacity even though some researchers noted regeneration of smooth muscles. The John Hopkins Hospital Report in 1910 after extensive investigation clearly indicated no sign of smooth muscle regeneration after six weeks. The interesting practical observation noted by all surgeons and confirmed by histologists, that rapidly growing capillaries in granulation tissue of healing wounds, the growth of smooth muscle in these capillaries cannot be denied. A brief interesting historical
account on observations on urinary bladder regeneration was described by Johnson et al\textsuperscript{19}. The medical literature of late 19\textsuperscript{th} and early 20\textsuperscript{th} century has seen many publications on regeneration capacity of urinary bladder after extensive urinary bladder resections\textsuperscript{17,20-24}. It was believed that urinary bladder has excellent regenerative capacity. A myth, which was removed by the detailed histological investigation of voiding pouch regenerated in place of urinary bladder after its extensive resection\textsuperscript{19,25}. The opinion probably prevailed due to formation of functional pouch in place of urinary bladder. Histology of this pouch revealed that the pouch consisted of granulation tissue connective tissue and collagen fibers etc. with a little or no smooth muscle formation but lined with transitional epithelium\textsuperscript{25}. Many reports showed human bladder regeneration without any smooth muscle regeneration\textsuperscript{27-32}. Johnson et al\textsuperscript{19}, expressed an interesting opinion that the bladder will not regenerate under any circumstances. Histological study of regenerated urinary bladder by Baker et al\textsuperscript{33} showed fibrous tissue laced with muscle bundles not continuous through bladder wall. Therefore they concluded that the muscle development was not an extension from urethra or ureter. They believed the origin of muscle was from multipotent mesenchymal–fibroblastic cell. Origin of smooth muscle tissue could be from blast cell, was the first bold opinion expressed in 1958\textsuperscript{33}. A wide variety of tissues are being used for augmentation cystoplasty including native tissues, homologous tissues from cadavers, heterogenous tissue from animals (bovine), and artificial synthetic polymers (silicone, polyurethane, teflon). These tissues found to be biocompatible and provide structural replacement but functional component of original tissue was not recovered\textsuperscript{34}. Augmentation cystoplasty is interesting but beyond the scope of this article. The associated complications of all these attempts, failed to get these procedures translated into clinical use.

Use of autologous peritoneal stem cells in regenerating abdominal wall aponeurosis in animals and subsequent successful use of this technique in 60 human patients of large incisional hernia\textsuperscript{8,12}, prompted us to undertake the regeneration of urinary bladder. The present research is an attempt at achieving this goal by using stem cells from appropriate adult tissues using embryological principles by which nature achieves morphogenesis in mother’s womb\textsuperscript{1,4,6-8}. It is well known that embryological morphogenesis of urinary bladder is from uro-genital sinus (UGS) after division of cloaca by uro-rectal septum (URS). During 4\textsuperscript{th} and 7\textsuperscript{th} week of development URS divides cloaca into anorectal canal and UGS. Upper larger part of UGS forms urinary bladder. Lower narrowed part forms urethra. Trigone of urinary bladder, is developed from Meso-Nephric Ducts and hence developed from germ layer mesoderm. Muscular and serosal walls of the organ are derived from splanchno-pleuric mesoderm\textsuperscript{5}. The rectum and urinary bladder have developed from cloaca, which is hind gut derived from endoderm. In other words, the cells which form hind-gut also forms urinary bladder in developing embryo. Therefore, endodermal stem cells from the hind gut-recs or-sigmoid colon, are used as per the hypothesis in this study, as embryologically both the structures are derived from endoderm layer and develop in contiguous regions during embryo development\textsuperscript{35}.

Patients of chronic peritonitis, have variety of abnormal tissue formation in abdominal cavity. Wide variety of metaplastic tissues range from smooth muscle, skeletal muscle, papillary projections, squamous nests, even calcification, osseous, cartilaginous or bony tissue formation, which were confirmed by pathological examination\textsuperscript{8}. This metaplasia is the result of chronic infection and altered chemical environment in the abdominal cavity. Therefore the metaplastic tissue formation is an altered commitment of cells under abnormal environment. If suitable environment and niche could be provided to these cells, the desired goal of directed regeneration could be achieved (vide hypothesis). Stem cells developed from a germ layer are present in adult tissues developed from the same germ layer. These cells possess the capacity to proliferate and maintain the differentiated cell population in their native tissues throughout the life span of an animal\textsuperscript{36}. The proliferation potential, makes these cells efficient in tissue regeneration through clonal growth\textsuperscript{11}. The embryological morphogenesis is facilitated by migration of appropriate cells to a place where the organ growth is essential, for example specific mesoderm cells migrate to kidney region for normal kidney morphogenesis in growing embryo. The cell migration, seen in developing embryo, is absent in adult body tissues. Therefore the desired tissues are surgically explanted to urinary bladder tissue system in the present study. The new environment/niche provided by the urinary bladder system would help in colonization of the explant tissue with the host tissues and undergo desired metaplasia\textsuperscript{34}. The cell surface receptors of colonized endoderm cells, respond to local tissue cytokines of bladder tissue system.
because both, host and colonized cells develop from the same germ layer and in contiguous embryological regions of developing embryo (hind gut)\textsuperscript{1,4}. In contrast to this observation, the stem cell membranes from jejunum, ileum, transverse colon, peritoneum etc. failed to contribute positive results (Table 3)\textsuperscript{3,4}.

The primitive reserve stem cells are present in the crypts of colon. The crypts are cylindrical structures present in both large and small intestines. The crypt tubes have stem cells in different stages of differentiation from base of the crypts to surface epithelium. These cells progress towards specialization for specific function. These cells migrate towards surface epithelium in crypt tube. Therefore the cells in the crypt tube from base to epithelial surface, are in different stages of differentiation. But the basal cells are pluripotent stem cells (PPSC) and are of endodermal origin\textsuperscript{9,16,37}. The paneth cells and PPSC remain in crypts. The PPSC are from 4\textsuperscript{th} to 6\textsuperscript{th} position of annulus of crypts above paneth cells (paneth cells are up to 3\textsuperscript{rd} position). The estimated number of clonogenic cells in the crypt is approximately 70-80 per crypt. A total of 16 functional stem cells lie above paneth cell zone beyond 3\textsuperscript{rd} annulus\textsuperscript{9}. These are resident stem cells while the other cells migrate towards surface and are capable of lineage differentiation. The niche (own anatomical location in intestine) provides the necessary environmental cues for tissue homeostasis. The epithelial cells obtained from recto-sigmoid colon have proliferation and differentiation capacity\textsuperscript{39}. The grafting experiments using intestinal cells reveal expression of $\alpha$-smooth muscle actin. Intestinal endoderm is capable of inducing $\alpha$-smooth muscle actin in intestinal mesenchyme and also in skin fibroblasts\textsuperscript{40}. Intestinal cell proliferation of intestinal crypts is also documented and studied in animal models\textsuperscript{38}. The existence of different stages of differentiated cells in intestinal crypts and the presence of stem cells at 3 and 4 annulus of basal intestinal crypts is well known. Hence surgically these cells are first exposed \textit{in situ} by dissecting them free from the surrounding layers of tissues in the isolated intestinal segment. The freshly isolated colonic membrane containing the stem cells of endodermal origin, was then transplanted on to the urinary bladder (see Material and Methods). Adult stem cells respond to the environmental cues dictated by the niche. In an undisturbed niche these cells are quiescent and non-proliferative, but when the niche is disturbed by injury or infection, they get activated and undergo proliferation and differentiation\textsuperscript{38-41}. Complete covering of transitional epithelium on grafted membrane was observed in all the animals in histological examinations after 60 days of observation period. The epithelium was normally and completely developed (Fig. 2c). The transitional epithelium is a fast growing epithelium. The covering of transitional epithelium has been observed on a variety of materials used in urinary tissue system, for example on autogenous, heterogenous, xenogenous, biodegradable and even on synthetic materials used

<table>
<thead>
<tr>
<th>Donor tissue source</th>
<th>Stem cell membrane</th>
<th>Germ layer origin</th>
<th>Relation with urinary bladder (in embryo)</th>
<th>Post-operative histology after 3 months</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>Intestinal submucosa with crypt base</td>
<td>Endoderm Germ layer</td>
<td>Not from Contiguous Embryo Region</td>
<td>Graft survives. Smooth Muscle Bundles + but scanty Fibrous bundles in Plenty. Patchy Transitional epithelium +</td>
<td>Incomplete regeneration of urinary Bladder wall unsatisfactory</td>
</tr>
<tr>
<td>Transverse Colon</td>
<td>Transverse submucosa Membrane With cryp Base.</td>
<td>Endoderm</td>
<td>Transverse Colon remotely Related to Urinary Bladder development but not in contiguous region</td>
<td>Smooth muscle bundles with Abundant fibrous bundles. Growth of Transitional epithelium + but patchy</td>
<td>Incomplete attempt At regeneration of urinary bladder wall.</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>Investing Layer mesoderm of Peritoneum From Abdominal wall</td>
<td>Different germ Layer source</td>
<td>Only growth of transitional epithelium seen. No smooth muscle growth observed.\textsuperscript{32,33}</td>
<td>Both peritoneum &amp; Urinary bladder From different germ Layers. No regeneration of bladder wall.</td>
<td></td>
</tr>
</tbody>
</table>

Table 3—Results of colonization of stem cell membranes from different sources with urinary bladder remnant after massive excision
for augmentation cystoplasty as stated above.\textsuperscript{42,46} Regeneration of transitional epithelium has been demonstrated even in xenografts of small intestinal submucosa stored for three months. Evidence of full thickness urinary bladder regeneration has also been demonstrated on animal studies by using small intestinal submucosa, collagen/polygalactic composite membrane.\textsuperscript{7,48} The smooth muscle regeneration was observed in the granulation tissue on the membrane and on bio-resorbable prosthesis, but the source of regeneration of smooth muscle remained unknown.\textsuperscript{49} Present results revealed formation of muscles after 60 days duration, \textit{de novo} all over the colonized membrane, suggesting regenerative changes in the membrane and not from the urinary bladder remnant. Similar changes were observed by Baker et al.\textsuperscript{33}. Such changes were not seen in histology of latter biopsies (90 days and later), as the growth of smooth muscle was uniform all over the grafted membrane. On H & E stain methods, both smooth muscle tissue and fibrous tissue were stained with eosine colour. On MTS stain the fibrous tissue stained green. The 60 days MTS stain revealed mixture of green and pink colour showing development of smooth muscles mingled with fibrous tissue (Fig. 3). Latter biopsies revealed increasing muscle tissue development. Some tissue bundles that neither stained green (fibrous) nor eosine (smooth muscle) and seen as pale tissue bundles (Fig. 3), were probably maturing smooth muscle bundles. These pale tissue bundles disappeared after biopsy sections of 90 days and after. The smooth muscle regeneration was uniformly seen. In tissue sections after three months post operative period, the smooth muscle formed in a uniform pattern as in normal bladder wall remnant as opposed to haphazard smooth muscle formation with collagen fiber interventions when porcine jejunum sub-mucosa was used in dogs.\textsuperscript{49,50} There was no stone formation in the present experiments, probably because the regenerated bladder wall was physiologically normal as compared to up to 80% stone formation observed when acellular matrix was used to augment the bladder capacity.\textsuperscript{4,41,43} Rejection, shrinkage, ulceration, or calcification of colonized membrane was not observed in any of the seven dogs. There was no infection, stone or diverticulum formation in the regenerated urinary bladder wall. The normal smooth muscle pattern resulted in normal voiding of urine in all the dogs. Voiding bladder pressure shows grossly unchanged readings despite barbiturate anesthesia. It is known that the barbiturates influence the maximum bladder pressure readings. For the same reason residual urine volume was not recorded to avoid false picture of urinary retention. However no residual urine was detected on post voiding catheterization in all the dogs. Near normal uro-dynamic studies (Table 1) indicated normal functional development. Regenerated urinary bladder showed normal capacity of urinary bladder as compared to pre-operative studies in the same animal (Table 1). All dogs showed near normal bladder pressure and voiding pressure and volume. The urine voiding after 90 days post operative period found to be normal (Table 1). Due to the absence of complications as mentioned above and near normal uro-dynamic studies, it is presumed that this technique may be a viable option for clinical use. But this procedure needs further standardization in primate models like monkeys, before it can be translated for clinical use in human beings. A critical and scientific aspect of the physiological phenomenon called “desired metaplasia”, has been explained in detail elsewhere.\textsuperscript{3,4} Body achieves homeostasis by repair and replacement of diseased or damaged cells during normal wear and tear of the body and this phenomenon is termed plasia.\textsuperscript{1-8,12,51,52} Chronic damage to cells due to chronic infection, irritation, radiation or persistently changed environment is protected by another phenomenon called metaplasia. This change of tissue or cells is abnormal to the anatomical abode where such change takes place. Metaplastic change is defensive mechanism of the body to resist further damage to the tissues or cells due to changed environment but tissue alteration is abnormal in that anatomical abode. Perfect organ and tissue formation in developing embryo is achieved by cellular migration to a site in embryo destined for that organ or tissue. In adult this capacity is lost. Therefore, when the stem cells from a tissue is surgically shifted to a different niche, they respond to the cues from the new environment in terms of alternate cell fate decisions. This change in cell or tissue, as per definition, is metaplasia but this change is needed and desired to adapt to the new anatomical abode. In other words, the transformation of stem cells in recto-sigmoid colon membrane at its intestinal anatomical abode, would have been termed as metaplasia. But at a changed anatomical abode of urinary bladder, the transformation is an adaptive response with a desired outcome. Hence it is termed as “desired metaplasia”. In neo-regeneration of
urinary bladder tissue, the stem cells of recto-sigmoid colon are surgically shifted to urinary bladder and the bladder wall neo-regeneration could be achieved. So far neo-regeneration of abdominal wall, ureter, fallopian tube, uterus, posterior urethra has been demonstrated. However, it is important to highlight experimental studies conducted on metaplasia of oral mucous membrane in context with desired metaplasia. Sweeney et al. excised skin of pinna of external ear of dog, and colonized oral mucous membrane on the cartilage. After two months the mucous membrane along with ear cartilage of pinna was excised and colonized in vivo with trachea after excising part of trachea. In first place in skin region the mucosa showed keratinization like skin. In second place in the region of trachea the same mucosa showed disappearance of keratinization and changed to ciliated columnar epithelium. Sweeney et al. explained this change as metaplasia. These events, can be explained as desired metaplasia. Oral mucous membrane is developed from two sources that is ectoderm of stomatodeum and endoderm of fore gut. When grafted in the skin region, where tissue cytokines (ectodermal) of extra cellular matrix (ECM) of skin were active. These stimulated cell surface receptors (CSR) of stem cells of oral mucosa, derived from endoderm germ layer source, to get keratinization effect due to specificity of action and reaction. In tracheal region developed from fore gut (endoderm origin) the oral mucosal cells developed from fore gut source (endoderm origin), CSR of cells of endoderm origin in composite graft, responded to ECM cytokines of endoderm origin in tracheal tissue system, resulting into ciliated columnar epithelium. Keratinization disappeared due to non-availability of stimulus of endoderm tissue cytokines in tracheal region. In metaplasia, it is observed that when the stimulus is removed or dissappers, the metaplastic transformation disappears. Therefore Sweeney’s experiment is a perfect example of desired metaplasia.

Conclusions

The urinary bladder can be regenerated by desired metaplasia of stem cells in colonic crypts in recto-sigmoid colon (hind gut) on colonizing them with urinary bladder tissue system. This is possible due to matching action and response of stem cells and host tissue, as both are derived from germ layer endoderm and grow in contiguous regions in developing embryo. The uro-dynamic studies indicated that neo-regenerated organ/tissue was compatible to normal urinary bladder wall capable of normal urine voiding. The urinary bladder function must be investigated in detail with quantitative and qualitative study of the regenerated smooth muscles in primate animal model like monkeys, before it is can be clinically translated for human use. Research in this direction is in progress.

References

10. Schwann T, Microscopical researches into the accordance of structure and growth of animals and plants (The Sydenham Society London) 1847.