Amniotic stem cells repair ureteric defect: a study to evaluate the feasibility of amniotic membrane as a graft in surgical reconstruction

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Abstract

Background Amniotic membrane is considered a promising procedure as a graft in the field of ophthalmology and skin reconstruction. It has been shown to decrease inflammation, fibrosis, elicit no host immune reaction and also has antibiotic actions.

Aim Recently, the amnion has been shown to contain cells capable of plasticity. Therefore, we felt its application could be extended to include surgical reconstruction.

Methods We used ureteric reconstruction to study the feasibility of the amnion as a graft in these situations. We induced different degrees of injury in the ureter of dogs and reconstructed the defect with amniotic membrane. In addition, we also studied the effects of supplying the graft with a source of blood supply.

Results The subjects were sacrificed after 6 weeks and histopathological examination showed impressive repair of the defect.

Conclusion The post reconstruction complications seen with present grafts were not seen with amniotic membrane. Therefore, amniotic membrane has produced noteworthy results and its potential should be further evaluated in order to be used as a cheap, readily available source of graft for the various surgical reconstruction procedures.

Keywords Amniotic membrane · Regenerative medicine · Stem cells

Introduction

The amnion is a thin, avascular membrane composed of an epithelial layer and an outer layer of connective tissue and is contiguous, over the umbilical cord, with the fetal skin. It is the innermost layer of the amniochorionic membrane which encloses the fetus. The amnion is rich in collagen and confers tensile strength, which resists the rupture of amniotic sac and also protects the fetus from mechanical injury.

On the 8–9th day of fertilization, the inner cell mass differentiates into the epiblast and the hypoblast. The amniotic cavity also develops in the inner cell mass and together with amnion forming cells (which are derived from the epiblast) constitutes a thin membrane; the amnion. The extra embryonic mesoderm, derived from the yolk sac, surrounds the amnion and lines the inner surface of the cytotrophoblast, giving rise to the amniotic and chorionic mesoderm [1].

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In 1910, Davis reported the use of fetal membrane as a surgical material in skin transplantation [2]. This was followed by the use of amniotic membrane in the treatment of skin wounds, burn injuries, chronic leg ulcers, prevention

of tissue adhesion in surgical procedures and also for ocular surface reconstruction [3–9]. Moreover, it has been reported that the human placental tissue acts as a source of feeder cells for the growth of primate embryonic stem cells [10].

Diagram 1 : (A) In group 1, 2 cm excision in ureter anterior wall was repaired using amnion graft only. Repairs were evaluated postoperatively on day 10 (subgroup 1A) and on 6 weeks (group 1B). (B) In group 2, 2 cm ureter segment excisions were repaired using amnion graft only. Repairs were evaluated postoperatively on day 10 (subgroup 2A) and on 6 weeks (group 2B)

Several studies have shown that amniotic epithelial cells (AEC) express some of the surface markers which are associated with embryonic stem cells (ESC), including SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. AEC also express pluripotent specific transcription factors; OCT-4 and Nanog [11]. Moreover, Tamagawa created a xenogenic chimera of human amnion cells with mouse ESC, *in vitro*. Chimeric aggregates were maintained and human contribution to all 3 germ layers was established [12], demonstrating that AEC are pluripotent. This was followed with further experiments which demonstrated *in vitro* differentiation of these cells into the 3 germ layers, including cardiac (mesoderm), neuronal, glial cells (ectoderm), pancreatic and hepatic cells (endoderm) and all have shown to be positive for their respective markers [11].

Furthermore, a population of multipotent cells, termed as placenta derived multipotent cells (PDMC), exhibit a mesodermal phenotype and are positive for embryonic stem cell markers; SSEA-4, TRA-1-61, TRA-1-8 and OCT 4. These cells have been shown to differentiate into osteocytes, chondrocytes and adipocytes, in addition to all the 3 germ layers. Moreover, several studies have reported that mouse placenta can serve as a source and as a functional niche for hematopoietic stem cells [15]. The above mentioned reports suggest that human amniotic epithelial cells (hAEC) share many stem cells characteristics. In addition to differentiating into multiple lineages, the hAEC have also been shown to create a microenvironment which can induce local cell activation and proliferation and also possess antifibrotic and antimicrobial activity [16].

These findings along with the success of amniotic grafts in the field of ophthalmology and skin reconstruction made us recognize the potential of amniotic membrane as a graft in surgical reconstruction. To test our hypothesis, we have decided to study the use of amniotic membrane as a graft in one of the most challenging scenarios in surgical practice, in terms of post reconstruction complications, namely the ureteric reconstruction. Due to the enormous demand to find a more suitable alternative graft for the above mentioned situation, we felt that amniotic membrane, along with its stem cell and wound healing properties would be an ideal graft in the above mentioned scenario. Therefore, we have decided to study the outcome of reconstruction procedures in the presence of amniotic membrane and also evaluate its feasibility as a graft.

In this study, we used amniotic membrane as a graft in ureteric reconstruction. Urologists are often faced with the need for ureteral replacement. The currently used ureteric substitute includes pedicled grafts from the ileum or the urinary bladder. Reconstruction of the ureter using these vascularised grafts restores the urinary tract continuity and integrity. However, some problems including; excessive mucus production, electrolyte imbalance, urinary reflux, dilation of upper urinary tract and chronic infection were encountered [17]. In addition, the insult to the intestine associated with obtaining the graft may itself lead to complications

[18]. Numerous synthetic materials including Vitallium, Tantalum, Teflon, Silicon and Gore-tex were used as urethral substitutes. Unfortunately, most of these synthetic materials were met with early failure due to infection, stone formation, migration and extrusion [19, 20].

The regenerative capacity of the ureter is remarkable [21]. Therefore, an ideal substitute should, therefore, be biodegradable, so that, it bridges the ureteral defect and remains until the natural host tissue regenerates and replaces it. Human amnion was used successfully as a biodegradable material in urinary bladder reconstruction in dogs [22]. Recently, preliminary study on two human cases demonstrated that reconstruction of long ureteral strictures with allogenic amniotic membrane seems to be a promising tool in the reconstruction of extensive ureteral strictures [23]. The aim of our work was to evaluate the amniotic membrane as a ureteral substitute in dogs.

Methods

Ureteric injuries

The study included 12 adult female mongrel dogs weighing 12–15 kg. Each dog was anaesthetized with sodium pentobarbital. The left ureter was exposed trans-peritoneally via a lower midline incision. In group 1 (six dogs), the anterior half of the ureteral wall was excised for a distance of 2cm in the middle third of the ureter. In Group 2, a 2cm segment in the mid-ureter was completely excised. Animal protocol was approved by ethical committee at Ain Shams University School of Medicine.

Human amnion

Human amnion was brought freshly from freshly delivered placentae and membranes, preferably after caesarian sections. Under aseptic conditions, the membranes were severed from the placenta, and washed thoroughly in sterile normal saline. The amnion was separated from the chorion, immersed in (1%) povidone iodine solution for 5 minutes and then washed again in normal saline. Following this, it was preserved in normal saline at 4°C, to be used within 48 hours.

Ureteric repairs

The amniotic membrane was folded once or twice on itself. In group 1, a suitable graft was cut and sutured to the edges of the ureteral defect with a continuous 5-zero polyglactin (vicryl) sutures. In group 2, a vascularised facial flap was prepared by cutting a rectangular part of the posterior rectus sheath with a thin layer of subjacent rectus muscle, about 3 by 4 cm, supplied by a branch of the inferior epigastric artery. The flap was mobilized preserving its blood supply. The inferior epigastric artery was mobilized by dividing all its other branches between ligatures to provide a long pedicle

Fig. 1 Ureter exposure. The ureter is slinged and posterior rectus sheath flap is mobilized

Fig. 2 Ureter repair procedure. The grafted segment of ureter is wrapped by the facial flap. The feeding artery to the flap is slinged

for the flap (Figs. 1 and 2). The 2 layered amnion graft was spread over the peritoneal surface of the flap. The amnion was then sutured longitudinally over a number 14 plastic catheter with a running 5-zero vicryl sutures, to form a tube, which was anastomosed to the 2 ends of the ureter with the same suture material. A 6 gauge feeding tube was placed as a stent and secured by anchoring sutures in the ureter and bladder. The fascial flap was sutured with few interrupted stitches in such a way so as to wrap the amniotic membrane graft and the anastomotic sites (Figs. 1 and 2). A rubber drain was brought out through a separate stab; the abdominal wound was closed with polypropylene. The wound and the drain were dressed with tie over bandages. A specially tailored jacket was fitted on to keep the wounds out of reach of the dogs. The drain was removed on the fifth day after operation. Gentamycin, 1mg/kg wt was given for 5 days.

Post-repair evaluation

Half the dogs in each group were assigned for re-exploration after 10 days (subgroups 1A and 2A), and the other half

after 6 weeks (Subgroups 1B and 2B). The left kidney and ureter were examined in situ. Nephroureterectomy was then done; removing the kidney and the ureter down to 5 cm below the site of the graft. The resected specimen was examined grossly and fixed in formalin. The grafted segment was totally processed routinely taking full thickness of the ureter and grafting material. Sections were prepared and stained with hematoxylin and eosin and Van Gieson stain, and were examined microscopically.

Results

All the dogs in group 1, except one, survived the operation and were well at the time of re-exploration. One dog died on the first postoperative day, but the cause of its death was not investigated. Three dogs were sacrificed after 10 days (subgroup 1A), and two were sacrificed after 6 weeks (subgroup 1B).

There were no early postoperative deaths in group 2. However, two dogs showed continued weight loss, deterioration of their general condition and failure to thrive and we had to sacrifice them after 6 and 13 days respectively. In the first dog (sacrificed on day 6), a large abscess was found at the operation site which had resulted in the destruction of the graft. The other dog (sacrificed on day 13) had pyonephrosis associated with obstruction of the ureter at the grafted site due to severe kinking. In addition, it was noticed that the stent has slipped downwards. The kink was found to be due to anchorage of the ureter by inadequately mobilized short fascial flap. Out of the remaining dogs, two were sacrificed after 10 days (subgroup 2A) and another two after 6 weeks (subgroup 2B).

On re-exploration after 10 days (subgroup 1A and 2A), the grafted site was readily identified. The upper urinary tract was found to be normal in all of these dogs. The grafted part of the ureter was easily mobilized by finger dissection. Microscopically, there was apparent generous proliferation of the transitional epithelium, which was lining the amniotic graft, forming a complete tube (Figs. 3A and B). There was early connective tissue regeneration underneath the transitional epithelium, growing from the edges of the defect (Figs. 3A and B). The amniotic graft was intact.

Six weeks after surgery (groups 1B and 2B), the left kidney and ureter were normal except in one dog from group 2B, which had mild hydronephrosis and hydroureter, due to kinking at the grafted site. There was mild to moderate periureteral fibrosis in the previously mobilized parts of the ureters. However, there was no difference in the degree of fibrosis in the grafted site and the ureter above and below it. Examination of the grafted site revealed full thickness regeneration of the ureter. In group 2B, the fascial flap was adherent to the ureteral wall. Microscopically, there was a well formed ureteric tube showing complete transitional

Fig. 3 Postoperative histological evaluation. (A): H&E section at 25 x showing regenerating transitional epithelium and sub-epithelial tissues over an intact amniotic graft 10 days after surgery. (B): H&E section at 100x showing multilayered transitional epithelium and sub-epithelial layer regenerating over an intact amniotic graft 10 days after surgery. AM: Amniotic membrane. TE: Transitional epithelium. M: Muscle layer.

epithelial lining, with regenerated submucosa and full thickness muscle layer, almost replacing the amniotic membrane graft. The epithelium was hyperplastic at the borders of the regenerated area.

Discussion

Much has been added to our knowledge of the ability of the ureter to regenerate. The ureter is known to regenerate over long areas as long as a strip of the wall is still bridging the gap [19]. The mucosa was found to be replaced within 1 week and the muscles within 6 weeks [20]. The situation is less favorable for complete circumferential defects. The muscle fibers could fill in approximately 2 cm over a complete gap, provided that the 2 ends are held together by a long stitch taken across this gap [21]. The remarkable natural ability of the ureter to regenerate is often marred by any adverse factors as urinary leakage, angulations and fixity of the ureter to underlying fascia or muscles, lack of an intact fatty sheath around the ureter, and failure of apposition of the ureteral ends. These factors lead to irregular growth of the epithelium, which may fail to form a tube, with the resultant failure of the muscle fibers to regenerate in a normal way and excess granulation tissue formation. Consequently, excessive fibrosis, stricture formation, sacculation, kinking, double lumen and severe delay in healing are often associated with natural regeneration of

ureteral defects, especially the complete circumferential ones [21].

In our study, the reconstruction of circumferential defects of the urethral was associated with obstruction and back pressure changes in one of the dogs. However, the obstruction was due to kinking of the ureter as a result of its anchorage by a short fascial flap, worsened by slipping of the stent, rather than due to fibrosis. This has occurred early in the series and was avoided in the subsequent experiments. Early regeneration of the transitional epithelium was noticed in our study, followed by a slower reconstitution of other constituent of the ureteral wall. It is known that normal transitional epithelial layer is an essential prerequisite for ureteral wall regeneration [23].

In addition, it has been reported that the human placenta can act as a source of feeder cells and have been used to support the undifferentiated growth of primate embryonic stem cells. Moreover, it has been observed that limbal epithelial cells grown on amniotic membrane have a much faster and a more mature basement membrane formation than normal wound healing models. It has been suggested that the amniotic membrane can serve as a feeder layer [10] and could have promoted basement membrane formation. It is expected that amnion may have acted in a similar way in our study by providing a suitable environment for basement membrane formation and aiding earlier epithelialization.

Rejection or early destruction of the grafts did not occur in our study, except in one dog, in which severe infection

has resulted in the destruction of the graft. Amnion is bio-compatible and has a natural low antigenicity which enables it to survive without immune rejection until the host tissues regenerate and replace it [24]. It was demonstrated that the AEC express no class II antigens and very low levels of the class Ib antigens HLA-E and HLA-G [24]. The HLA-G molecule displays at least four inhibitory functions relevant to immune responses: first, it can bind directly to inhibitory receptors found on NK cells and other leukocytes [25,26]; secondly, it possesses the appropriate leader peptide for binding to HLA-E, which will in turn inhibit NK cells via their CD94/NKG2 receptors [27]; thirdly, as shown recently, soluble forms of HLA-G produced by placental cells induce apoptosis of activated CD8⁺ T cells [28]; finally, HLA-G can inhibit CD4⁺ T cell proliferation [29].

Additional experiments have shown that fragments of amniotic membrane exert immunosuppressive properties in mixed lymphocyte reactions [30].

Amnion possesses several other characteristics that make it suitable for ureteral bio-prosthesis. It is readily available, obviating the need for processing and storage at practically no cost. Amnion derives its nutrients by diffusion, thus can be used as a free graft. We found that the amniotic membrane was easily handled, could hold the sutures well. The sutured graft was water tight. Similar observations were reported in other studies [14, 31]. Finally we would like to add that AEC do not raise ethical concerns like embryonic stem cells (ESC) and they have not been shown to induce tumors after transplantation. Moreover, amnion is a superior source of adult stem cell than the bone marrow. It has been reported that from day 7 of culture onwards, the cellular yield of amniotic mesenchymal stem cells obtained from a small area (4 cm²) of amniotic membrane was considerably higher than the yield obtained from the same number of bone marrow derived cells [32]. Amniotic mesenchymal cells also expressed Oct-4 mRNA at higher levels than the bone marrow mesenchymal cells; this is noteworthy, as the Oct-4 transcript is normally found in totipotent embryonic stem cells and the expression of the gene is down regulated during differentiation [33].

Conclusion

As shown in this study; the amniotic membrane can act as an ideal graft, which initially acts as a skeleton for the regeneration of the normal tissue then gradually biodegrades. Moreover, amniotic membrane grafting conserves the normal anatomy and physiology of the ureter. Amniotic membrane is also readily available, obviating the need for processing and storage at practically no cost. Furthermore, it can handle sutures well, of low cost, ethical, causes no host reactions such as immune rejection and decreases inflammation, fibrosis and infection. Therefore, amniotic membrane is an excellent source of graft as seen above.

Since, there are few studies regarding amniotic membranes as a graft in reconstruction surgery, results should be further evaluated; in terms of long term follow up. On the other hand, studies aiming at increasing the efficacy as well as identifying factors supporting amniotic graft such as blood supply in our study are needed. In conclusion, amniotic membrane possesses several valuable properties which make it a likely candidate for an alternative source of graft for reconstruction surgery.

Conflict of interest The authors do not have any disclosable interest.

References

- 1 Toda A, Okabe M, Yoshida T, Nikaido T (2007) The Potential of Amniotic Membrane/Amnion-derived cells for Regeneration of Various Tissues. *J Pharmacol Sci* 105(3): 215–228
- 2 Davis JW (1910) Skin transplantation with a review of 550 cases at the Johns Hopkins hospital. *Johns Hopkins Med J* 15:307–396
- 3 Trelford JD, Trelford-Sauder M (1979) The amnion in surgery, past and present. *Am J Obstet Gynecol* 134:833–846
- 4 Colochio G, Graham WP 3rd, Greene AE, Matheson DW, Lynch D (1974) Human amniotic membrane as a physiologic wound dressing. *Arch Surg* 109: 370–373
- 5 Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, Hsi BL (1980) Human amnion as an adjunct in wound healing. *Lancet* 1:1156–1158
- 6 Subrahmanyam M (1995) Amniotic membrane as a cover for microskin grafts. *Br J Plast Surg* 48:477–478
- 7 Gruss JS, Jirsch DW (1978) Human amniotic membrane: a versatile wound dressing. *Can Med Assoc J* 118: 1237–1246
- 8 Ward DJ, Bennett JP (1984) The long-term Results of the use of human amnion in the treatment of leg ulcers. *Br J Plast Surg* 37:191–193
- 9 Ward DJ, Bennett JP, Burgos H, Fabre J (1989) The healing of chronic venous leg ulcers with prepared human amnion. *Br J Plast Surg* 42:463–467
- 10 Miyamoto K, Hayashi K, Suzuki T, Ichihara S, Yamada T, Kano Y, Yamabe T, Ito Y (2004) Human Placenta Feeder Layers Support Undifferentiated Growth of Primate Embryonic Stem Cells. *Stem Cells* 22:433–440. *Cells* 2007
- 11 Miki T, Lehmann T, Cai H et al. (2005) Stem Cell Characteristics of Amniotic Epithelial Cells. *Stem Cells* 23: 1549–1559
- 12 Gekas C, Dieterlen-Lievre F, Orkin SH et al. (2005) The placenta is a Niche for Hematopoietic Stem Cells. *Dev Cell* 8:365–375
- 13 Tachibana M, Nagamatsu GR, Addonizio JC (1985) Ureteral replacement using collagen sponge tube grafts. *J Urol* 133:866
- 14 Fishman IJ, Flores FN, Brantly Scott F, Spjut HJ, Morrow B (1987) Use of fresh placental membranes for bladder reconstruction. *J Urol* 138:1291
- 15 Lord JW Jr, Eckel JH (1942) The use of vitallium tubes in the urinary tract of the dog. *J Urol* 48:412

- 16 Lubash (1947) Experience with tantalum tubes in the re implantation of the ureters into the sigmoid in dogs and humans. *J Urol* 57:1010
- 17 Solomon A, Wajngarten M, Alviano F, Anteby I, Elchalal U, Pe'er J, Levi-Schaffer F (2005) Suppression of Inflammatory and Fibrotic Responses in Allergic Inflammation by the Amniotic Membrane Stromal Matrix. *Clin Exp Allergy* 35(7):941–948
- 18 Lapidus J, Caffery EL (1955) Observations on Healing of Ureteral Muscle: Relationship to Intubated Ureterotomy. *J Urol* 73:47
- 19 Trelford JD, Trelford-Saunders MV (1979) The amnion in surgery, past and present. *AM J Obstet Gynecol* 134:833
- 20 Weaver RG (1985) Ureteral regeneration: Experimental and clinical, Part III. *J Urol* 79:31
- 21 Lapidus J, Caffery EL (1955) Observations on Healing of Ureteral Muscle: Relationship to Intubated Ureterectomy. *J Urol* 73:47
- 22 Hinman F Jr., Oppenheimer, R (1956) Smooth muscle regeneration in repair of experimental ureteral defects: the significance of double lumen. *J Urol* 75:428
- 23 His BL, Yeh CJ, Faulk WP (1982) Class antigens of the major histocompatibility complex on cytotrophoblast of human chorion leave. *Immunology* 53:621
- 24 Koziak A, Salagierski M, Marcheluk A, Szcześniewski R, Sosnowski M (2007) Early Experience in Reconstruction of Long Ureteral Strictures with Allogenic Amniotic Membrane. *Int J Urol* 14(7):607–611
- 25 His BL, Yeh CJ, Faulk WP (1982) Class antigens of the major histocompatibility complex on cytotrophoblast of human chorion leave. *Immunology* 53:621
- 26 Kubo M, Sonoda Y, Muramatsu R, Usui M (2001) Immunogenicity of Human Amniotic Membrane in Experimental Xenotransplantation. *Invest Ophthalmol Vis Sci* 42:1539–1546
- 27 Allan DS, Lepin EJ, Braud VM, O'Callaghan CA, McMichael AJ (2002) Tetrameric complexes of HLA-E, HLA-F, and HLA-G. *J Immunol Methods* 268:43–50
- 28 Colonna M, Samaridis J, Cella M, Angman L, Allen RL, O'Callaghan CA, Dunbar R, Ogg GS, Cerundolo V, Rolink A (1998) Human Myelomonocytic Cells Express an Inhibitory Receptor for Classical and non Classical MHC class I molecules. *J Immunol* 160:3096–3100
- 29 Llano M, Lee N, Navarro F, Garcia P, Albar JP, Geraghty DE, Lopez-Botet M (1998) HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: Preferential Response to an HLA-G-derived Nonamer. *Eur J Immunol* 28:2854–2863
- 30 Fournel S, Aguerre-Girr M, Huc X, Lenfant F, Alam A, Toubert A, Bensussan A, Le Bouteiller P (2000) Cutting edge: Soluble HLA-G1 triggers CD95/CD95 Ligand Mediated Apoptosis in Activated CD8+ Cells by Interacting with CD8. *J Immunol* 164:6100–6104
- 31 Bainbridge DR, Ellis SA, Sargent IL (2000) HLA-G Suppresses Proliferation of CD4 (+) T-lymphocytes. *J Reprod Immunol* 48:17–26
- 32 Ueta M, Kweon MN, Sano Y, Sotozono C, Yamada J, Koizumi N, Kiyono H, Kinoshita S (2002) Immunosuppressive Properties of Human Amniotic Membrane for Mixed Lymphocyte Reaction. *Clin Exp Immunol* 129: 464–470
- 33 Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M (2007) Term Amniotic Membrane is a high throughput source for Multipotent Mesenchymal Stem Cells with the ability to differentiate into Endothelial Cells in vitro. *BMC Dev Biol* 7:11
- 34 Loh YH, Wu Q, Chew JL et al. (2006) The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet* 38:431–440