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Full Length Article

Structural and functional renovation of urinary bladders after amniotic membrane implantation in dogs

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Abstract Amniotic membrane as a biocompatible and permi-selective immune barrier was harvested from full-term pregnant bitches and cryopreserved by using Dulbecco's modified Eagle's medium (DMEM) or Minimal essential medium (MEM) 50% medium in glycerol 50% at temperature below freezing −80 °C. A defined 3 cm × 3 cm patch in the craniodorsal surface of the bladder was excised and then autografted in 15 apparent healthy mongrel dogs. In the place of excised patches of 27 apparent healthy mongrel dogs, double layers of 4 cm × 5 cm cryopreserved amniotic membranes were implanted. The results of plain and contrast radiography, ultrasonography, blood and urine analysis and histopathology confirmed that urinary bladder surgical defect that replaced by amniotic membrane implant, proved successful revival by normal structural, functional and contractile layers without any growth abnormalities throughout three months period. The successful implantation model of amniotic membrane will encourage its use on human beings for renovation in the surgical management of bladder cancers and restoring damaged or diseased urinary bladders.

1. Introduction

Treatment of the urinary bladder diseases comprised congenital abnormalities, cancer, trauma, infection, inflammations and iatrogenic injuries [1,2]. Unfortunately, the previous attempts of urinary bladder substitutes using both natural and synthetic biodegradable materials had been failed due to mechanical, structural, functional or biocompatibility problems [3–6].

It was regarded to use the amniotic membrane without any additional component of the placenta due to the chorion pro-

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voked revascularization and inflammatory reaction in the host tissue which sooner or later caused a rejection phenomenon [7].

The idea of substitution was to maintain bladder wall integrity, enhance the bladder capacity and decrease intravesical pressure. Amniotic membrane as a substitute for the urinary bladder of dogs was beneficial in regenerative therapy. The biocompatible substitute biomaterial was able to serve as a scaffold for the regeneration of all layers of the urinary bladder [8].

The present study aimed to prove the efficiency of amniotic membrane implantation as urinary bladder wall substitute and hoping that it will be an acceptable and good model for developing human surgery in this field and his welfare.

2. Materials and methods

The current research was carried out on 27 healthy mongrel dogs for allografting, 15 dogs for autografting and 5 dogs as a control group, of both genders, aged from 2 to 5 years and weighed from 15 to 20 kg. Further 5 full-term pregnant bitches were verified by ultrasonography examination as stated by [9,10]. These pregnant dogs were subjected to selective laparo-hysterotomy for harvesting, then preserving and banking of the amniotic membrane. The obtained placentas were immersed in normal saline, containing 100 U/ml penicillin and 0.2 mg/ml streptomycin (Pen & Strept®; Norbrook Netherland) and 0.025 mg/ml amphotericin B according to [11,12]. The amniotic membrane was peeled off chorion and molded on a sterile sheet of nitrocellulose, then preserved in temperature below freezing −80 °C [12,13].

The amniotic membrane was then harvested by blunt dissection from chorion. The epithelial covering was marked by external knot of mersilk stitch which was laid on the surface of the amniotic membrane. Under strict sterilization serial washing of the membranes in sterile Petri-dishes contained 20 ml normal saline which 100 U/ml penicillin, 0.2 mg/ml streptomycin and 0.025 mg/ml amphotericin were added. During washing the individual membrane was finger rubbed and squeezed of the blood vessels which were done gently to remove excessive blood clots, then kept in a container containing normal saline with antibiotics and antifungal additives for a period of two hours cooling in a refrigerator. This preparation was made according to [12,13].

For long-term storage beyond one year; the amniotic membranes were transferred to laboratory in a sterile plastic bag in an ice bucket. Under laminar-flow, the membrane was rinsed several times with normal saline contained antibiotics and antifungal mixture. During immersion the amniotic membranes were molded on a sterile sheet of nitrocellulose membrane NC2 (SERVA-Germany; 5 sheets sized 20 cm × 20 cm with 0.2 μm pores size). The molded membrane sheet was cut into 4 cm × 5 cm pieces as it acquired adhesiveness with the membrane, caution was paid to let the epithelial layer up side. Each cut piece of the molded membrane sheet was put in a plastic sterile Petri-dish contained DMEM 50% (Dulbecco's modified Eagle's medium, Sigma Company) or MEM 50% (Minimal essential medium, Sigma Company), both medium were in a ratio with glycerol 50% (1:1) with antibiotics and antifungal additives. The harvested membranes were preserved in temperature below freezing −80 °C by using NuAire-Ultra Low Freezer (USA). The preserved membranes were used as a graft subsequent to thawing in room temperature. This preservation was made with slight modification according to [12,13].

Laparo-cystoplasty by autograft and Allograft: The selected dogs were subjected to laparo-surgeries under the effect of general intravenous anesthesia. Premedication was adopted by injecting Atropine sulphate (Atropine sulphate 1%®, Adwia Co., SAE, Egypt 0.05–0.1 mg/kg b wt) and xylazine (Xyla-Ject 2%®, Adwia Co. SAE, Egypt 1 mg/kg b wt.), then anesthesia was induced using ketamine HCL (Ketamine®, Sigma-Tec, Egypt 10–15 mg/kg b wt.) and maintained by inhalation of gas mixtures which composed of oxygen and isoflourane in a ratio of 1: 0.5–1.5 with the help of intermittent positive pressure ventilation at 16 time/minute that derived from SurgiVet inhalation apparatus according to [14]. A ventral midline approach in females and ventral midline-paramedian approach in males were adopted for partial laparocystectomy. In the autografted group a defined 3 cm × 3 cm patch in the dorsal surface of the bladder was excised and immediately re-implanted in situ by water tight continuous sutures using vicryl 3/0 (Surgi Sorb®, Suture Ltd., UK). In the allografted group a defined 3 cm × 3 cm patch in the dorsal surface of an empty and contracted bladder was resected and replaced by double layers of 4 cm × 5 cm preserved amniotic membranes. The first layer was sutured to the bladder wound with simple continuous sutures using vicryl 3/0 and the other layer was applied using 3M Vetbond; *n*-butyl cyanoacrylate surgical glue (Fig. 1).

Following augmentation of the amniotic membrane, the augmented bladder was filled with 20-30 ml normal saline via a previously applied polyethylene urinary catheter to test exactness of the graft suturing. Intra abdominal Pen & Strept® antibiotic was applied, prior to opposing the laparotomy wound layer by layer using vicryl 2/0. Wounds were cared daily and the skin sutures were removed 10 days postoperative. Systemic antibiotic ceftriaxone® (ceftriaxone sodium 50 mg/kg, IM, Novartis Pharma SAE, Egypt) was daily injected. Dogs were provided with daily balanced diet and fresh clean water ad libitum.

Venous blood and clean catch urine samples were collected at intervals; before surgery, 1, 2 and 3 months postoperatively. BUN and creatinine were estimated in serum and urinalysis included color and turbidity, specific gravity, pH, chemical components and microscopic examination of the cells and solid sediment in the urine were evaluated.

Ultrasonography scanning was applied using B-mode transducers 5.0 MHz convex and 7.5 MHz micro-convex according to [10,15].

Conventional and contrast radiography were also performed; urografin 76% and air for double contrast and air alone for negative contrast cystogram were employed. Radiographic views were undertaken for each dog included lateral, ventrodorsal and two oblique projections according to [16].

Nine dogs of allograft group and five dogs of autograft group were traced and euthanatized after 1, 2 and 3 months. Humane euthanasia and postmortem examination were completed and resection of dorsal surfaces of the bladders including the graft took place.

The resected specimens fixed in formol saline 10% then washed, dehydrated, cleared, embedded in paraffin and sectioned then stained according to [17]. H&E stained sections were examined microscopically.