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Decellularized human fetal intestine as a bioscaffold for regeneration of the rabbit bladder submucosa

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ABSTRACT

Purpose: We aim to report a method to create a natural acellular scaffold from human fetal small intestine for augmentation cystoplasty in rabbits.
Methods: Fetal intestines were decellularized by immersion in a hypotonic solution. The success of this protocol was evaluated by histological analysis, scanning electron microscopy and measurement of collagen and sulfated glycosaminoglycan of the acellular tissues. Eight mature rabbits were selected and acellular scaffolds were implanted on the exposed urothelium. Urodynamic studies and cystography were performed after six months. At 14, 120 and 180 days animals were sacrificed and augmented bladders were resected.
Results: Histological analysis revealed formation of muscular layer and blood vessels in implanted scaffolds similar to normal bladder. These findings demonstrate the effective seeding of scaffold by host bladder cells. The tissue architecture of recellularized scaffold was similar to the native bladder.
Conclusions: Fetal intestine acellular matrix could be an exceptional scaffold for bladder augmentation cystoplasty and may pave the road for future studies in order to be used for clinical application.

Despite dramatic advances in management of bladder disorders, augmentation cystoplasty still plays a major role in treatment of severe bladder dysfunction when other options fail. The choice of grafting tissue however, still remains one of the most challenging issues in reconstructive urology. Currently, gastrointestinal segments are often employed for bladder augmentation, but complications like stone formation, metabolic abnormalities and secondary malignancies commonly complicate this procedure [1–3]. As a result, investigators have continuously sought to find novel materials and techniques for bladder augmentation.

Tissue engineering offers new hopes for treatment of patients needing organ replacement or repair. Biological scaffolds derived from tissue extra-cellular matrix (ECM) have been successfully used for a variety of tissue engineering applications [4]. ECM is composed of a mixture of structural and functional proteins, all arranged in a three-dimensional architecture. The main protein of ECM is collagen, representing greater than 90% of dry weight of ECM in most tissues and organs [4,5]. ECM contains proteins that are conserved during evolution and thus ECM grafts are usually well tolerated even by xenogeneic recipients [5]. Biological scaffolds may provide ideal media for tissue engineering since they are biocompatible, bioabsorbable, nonimmunogenic, and are able to support cell attachment and growth of reseeded cells in vivo.

Fetal tissues have a high capability for regeneration due to high levels of growth factors in their ECM. For instance, various clinical and experimental studies have used the amniotic membrane as a biological matrix and showed that the transplantation of amniotic membrane modulates angiogenesis [6] and decreases tissue fibrosis [7]. Additionally, fetal tissues like skin are known for high regenerative capacity with minimal scar formation in the process of wound healing [8]. Several factors may mediate these processes that include: high levels of growth factors; distinct biomechanical characteristics; anti-inflammatory and immunomodulatory properties; and unique ECM profile [8]. However, most studies focusing on regenerative capacity of fetal tissues are confined to skin and eye, with fewer investigating this issue in urogenital and colorectal tissues. Additionally, a gap of knowledge exists whether decellularized fetal tissues can be safely used with acceptable outcomes for different tissue engineering applications.

Given the aforementioned evidence, we aimed to use a novel decellularized fetal matrix for augmentation cystoplasty. We used segments of decellularized human fetal intestine and grafted it to rabbit bladder. We also performed histological and functional studies to evaluate the success of our method.

1. Materials and methods

The ethical committee of Tehran University of Medical Sciences approved the protocol of this study on aborted human fetuses.

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1.1. Human fetal intestine preparation

Three stillborn fetuses, all under thirteen weeks of gestational age, underwent autopsy with a midline excision (Fig. 1). All fetuses had normal gastrointestinal tract with no anomalies. The small intestine was dissected free and removed from pylorus to ileocecal valve. Fat was removed from the intestines and the specimens were carefully irrigated with normal saline.

1.2. Decellularization process

The following protocol was used to decellularize the harvested organs:

At room temperature the intestinal segments were washed with phosphate buffer saline (PBS). The human fetal intestines were cannulated and the tissues were perfused in a 0.5% solution of sodium dodecyl sulfate (Sigma–Aldrich, Belgium) for 12 h to remove cell membranes. Finally, the resultant scaffolds were once again rinsed with PBS to remove any toxic material. The scaffolds were preserved in an antibiotic solution (combination of penicillin, ceftriaxone and amphotericin) at 4 °C until the time of surgery.

1.3. Acellular scaffold characterization

To confirm complete decellularization, scaffolds were assessed with hematoxylin and eosin (H&E) staining after fixation in 10% neutral buffered formalin solution, dehydration in graded alcohol, and sectioning. Picro-Sirius red staining was used to evaluate the presence of collagen fibers in acellular construct. Type I collagen fibers are seen as thick, strongly birefringent, yellow orange fibers, while type III fibers are visualized as thin, weakly birefringent, greenish fibers. Russel-Movat Pentachrome (Sigma-Aldrich, Belgium) staining was used to differentiate connective tissue constituents (collagen: yellow; elastin: black; muscle: red; mucin: blue; and fibrin: bright red). Collagen and sulfated-glycosaminoglycan (S-GAG) content of tissues were quantified using the Sircoland Blyscan assay kits (Biocolor Ltd., UK), respectively.

Natural and decellularized human fetal intestine specimens were also assessed using scanning electron microscopy (SEM) by lyophilizing the decellularized tissue. Samples were examined using field emission SEM (FE-SEM; JSM-6340F, JEOL, Tokyo, Japan).

1.4. Surgical technique

Eight male rabbits were chosen for the surgery. Each rabbit was fasted overnight and given a pre-operative dose of intravenous cefazolin. Rabbits were anesthetized by intramuscular injection of ketamine (25–30 mg/kg) and xylazine (5 mg/kg). Urinary bladder was exposed through a midline lower abdominal incision. Upon removal of perivesical fat, the seromuscular layer was dissected and separated until a wide-mouth herniation of bladder mucosa was created. Then an approximately 5 cm^2 acellular scaffold was used to cover the resulting herniation (Fig. 2). 6–0 Vicryl sutures were used and care was taken not to penetrate the inner luminal surface. The water-tightness of suture lines was tested by instilling saline into the bladder. Enrofloxacin was administered for all rabbits postoperatively.

1.5. Evaluation of cell seeding in implanted scaffolds

At 14, 120 and 180 days animals were sacrificed and tissue biopsies were taken for histologic analysis. Tissue slides were stained with H&E to assess the histologic changes in tissues. To further analyze scaffold reseeding, we used immunohistochemistry (IHC). After fixation, samples were dehydrated in xylene and 100% ethanol and subsequently embedded in paraffin. IHC staining was performed by overnight incubation at room temperature using a previously described method [9]. Anti-smooth muscle antibody (anti-SMA), anti-vimentin, and anti-cytokeratin were used for labeling of smooth muscle cells, mesenchymal cells, and urothelium, respectively.

1.6. Cystography and cystometry

Bladders were assessed radiologically by cystography before surgery and six months afterwards. The rabbits were anesthetized and a 5-Fr feeding tube was advanced through the urethra into the bladder. The contrast media was injected through the catheter and cystography was performed.

The cystometric parameters were measured for three rabbits, six months after the surgery. Three other animals were used as control. Animals were anesthetized and using a 5-Fr transurethral catheter the rabbit bladders were emptied and then filled with saline solution at a constant rate prior to the experiment. A rectal balloon catheter was used to measure the intraabdominal pressure simultaneously. The vesical pressure was measured using a MMS urodynamic system (Laborie Medical Technologies Inc., Mississauga, ON, Canada).

1.7. Statistical analysis

Statistical analysis was performed using SPSS®, v19 (IBM SPSS Statistics, IBM Corporation. Chicago, IL, USA). Data are presented as mean ± SE. Biochemical analysis was performed using paired t-test to compare the values before and after decellularization. Independent

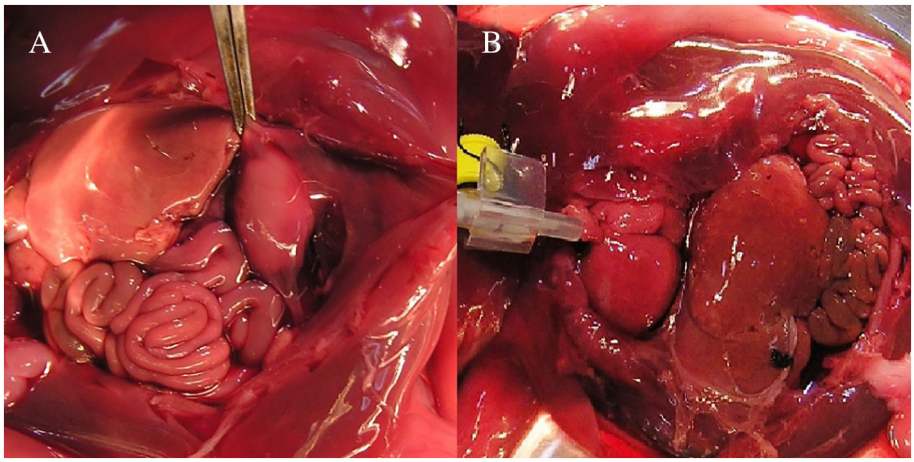

Fig. 1. The human fetal intestine was exposed (A), and the aorta was cannulated to wash out blood from the body (B).

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