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Bladder muscular wall regeneration with autologous adipose mesenchymal stem cells on three-dimensional collagen-based tissue-engineered prepuce and biocompatible nanofibrillar scaffold

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Abstract *Objective:* Tissue-engineered prepuce scaffold (TEPS) is a collagen-rich matrix with marvelous mechanical properties, promoting in vivo and in vitro tissue regeneration. In this study, adipose-derived mesenchymal stem cells (ADMSCs) were used to seed TEPS for bladder wall regeneration. Its potential in comparison with other materials such as polyglycolic acid (PGA) and nanofibrous scaffolds were evaluated.
Materials and methods: Rat ADMSCs were cultured and seeded into prepared TEPS. A synthetic matrix of electrospun nanofibrous polyamide was also prepared. Sprague Dawley rats ($n = 32$) underwent bladder wall regeneration using (a) TEPS, (b) TEPS + PGA, (c) TEPS + nanofibrous scaffold, and (d) ADMSC-seeded TEPS, between bladder mucosa and seromuscular layer. Animals were followed for 30 and 90 days post implantation for evaluation of bladder wall regeneration by determining CD31/34 and SMC α-actin. Cystometric evaluation was also performed in all groups and in four separate rats as sham controls 3 months postoperatively.

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Results: Histopathological analysis showed well-organized muscular wall generation in ADMSC-seeded TEPS and TEPS + three-dimensional (3D) nanofibrous scaffold without significant fibrosis after 90 days, while mild to severe fibrosis was detected in groups receiving TEPS and TEPS + PGA. Immunohistochemistry staining revealed the maintenance of $CD34^+$, $CD31^+$, and α-SMA in ADMSC-seeded TEPS and TEPS + 3D nanofibrous scaffold with significantly higher density of $CD34^+$ and $CD31^+$ progenitor cells in ADMSC-seeded TEPS and TEPS + 3D nanofibrous scaffold, respectively.
Conclusions: This work has crucial functional and clinical implications, as it demonstrates the feasibility of ADMSC-seeded TEPS in enhancing the properties of TEPS in terms of bladder wall regeneration.

Introduction

Tissue engineering has shown impressive outcomes regarding bladder wall reconstruction during the previous decade. Several natural and synthetic biodegradable scaffolds have been evaluated for bladder wall regeneration. However, controversy still exists in terms of clarifying the best scaffold for bladder tissue reconstruction for proper regeneration of all three layers of urinary bladder. Primary cultures of bladder urothelial and smooth muscle cells (SMCs) are usually obtained by surgically invasive methods via harvest of a part of patients' bladder [1], making it impractical for routine clinical use. These cell cultures are usually used in bladder wall regeneration after 6–8 weeks of cell expansion for the purpose of achieving a cell mass capable of being seeded on biodegradable molds [2]. Regarding the fact that the accessibility of healthy bladder tissue in patient population with malignant and pathological bladders is notably restricted, the potentiality of urothelial and SMCs has been limited to date. Tissue engineering critics have also pointed out that the traditional bladder cell harvest procedures are surgically invasive, and the prolonged cell expansion times are expensive [3]. As a consequence, application of an alternative source for harvesting SMCs seems inevitable.

Adipose tissue is akin to bone marrow stroma, and both originate from embryonic mesoderm and contain pluripotent progenitor cells with potentiality of multipotency and self-renewal [4,5]. Within adipose tissue, adipose-derived mesenchymal stem cells (ADMSCs) are more immune-compatible and their harvest, isolation, and expansion is much easier than bone marrow-derived mesenchymal stem cells (BMMSCs) [6,7]. The capability of ADMSCs to differentiate into functional and contractile SMCs may provide a novel approach for bladder wall regeneration.

The goal of this study was to explore the feasibility of utilizing ADMSCs and nanofibrous scaffolds for bladder reconstruction in a rat model. In the present study, the results of bladder muscular wall reconstruction have been compared in four different biomaterials including tissue-engineered prepuce scaffold (TEPS), TEPS + polyglycolic acid (PGA), TEPS + three-dimensional (3D) nanofibrous scaffold, and ADMSC-seeded TEPS in rabbit models.

Materials and methods

Preparation of TEPS

The decellularization protocol of the present study was applied to prepuce obtained during circumcision of children under aseptic conditions. Prepuce tissue was washed with normal saline and the mucosa was removed using Meezan's modified method [8]. To separate the epidermis from the dermis, the prepuce was incubated with its mucosal side downwards in a petri dish containing 1 M NaCl. After incubation at 38 °C for 48 h, the Petri dish was returned to the laminar hood for de-epidermization. The epidermis smoothly was removed from the dermis using forceps. Then, the prepuce was rinsed with 20 mL of Hanks' balanced salt solution (HBSS) on a rotator at 60 r.p.m. for 10 min at room temperature. The prepuce was then immersed in 20 mL of decellularizing solution (1% sodium dodecyl sulfate (SDS) in HBSS) and placed on a rotator at 60 RPM for 90 min at room temperature. The prepuce was rinsed in 1% triton X-100 solution for 20 min then washed in phosphate buffer saline (PBS) for 10 min. The process was continued by treating the prepuce with a mixed solution containing 0.05% trypsin/0.02% EDTA and incubating at 38 °C for 30 min. Afterwards, the prepuce was washed in 20 mL of PBS at 80 RPM for 20 min. The decellular scaffold was stored in a well-closed container; filled with antibiotic-containing PBS (100 U/mL penicillin-G, 100 U/mL streptomycin (Gibco) and amphotericin B) at 4 °C for future use.

Characterization of scaffolds

To ascertain the maintenance of extracellular matrix (ECM) structure, scanning electron microscopy (SEM) was performed. TEPSs were placed in a 2.5% solution of glutaraldehyde for 1.5 h at 4 °C then placed in PBS for 90 min at 4 °C. Subsequently, they were placed in a graded ethanol–water series. A Gatan ion beam coater was applied to coat the dehydrated specimens (approximately 2 nm thick).

To measure the biomechanical properties of TEPS and compare it with natural bladder, a tensile-test device (Zwick/Roell, Model: Hct 400/25, Germany) was applied. A mounting uniaxial force with an acceleration rate of 2 mm/min was employed for both tissues, which had been