Introduction

Cell-based tissue engineering offers an alternative technique for long segmental urethral reconstruction. The goal of this study was to determine whether urothelial cells (UC) and smooth muscle cells (SMC) differentiated from urine-derived stem cells (USC) could be used to form urethral tissue when seeded on a 3D porous small intestine submucosa (SIS) matrix for potential use in urethral tissue engineering.

Materials and Methods

USC were harvested from 12 urine samples from 4 healthy individuals. The cells were expanded in culture and induced to differentiate into either UC with epidermal growth factor (EGF, 30 ng/ml) or SMC with myogenic growth factors (PDGF-BB 5 ng/ml, and TGF-β 2.5/ml) for 2 weeks. Fresh SIS scaffolds derived from pigs were treated with 5% peracetic acid (PAA) and the porosity of the matrix was assessed using scanning electron microscopy. Differentiated USC (to UC and SMC) were seeded onto the mucosal side of the SIS in a layered co-culture fashion. As a control, UC and SMC from bladder tissue were seeded onto the SIS in the same manner. Seeded SIS constructs were cultured under dynamic culture conditions for one week and then implanted subcutaneously into athymic mice. Prior to implantation and one month afterwards, cell growth, cell-matrix infiltration, and urothelial and smooth muscle phenotypes were evaluated using histological and immunocytochemical techniques.

Results

Treatment with 5% PAA created a highly porous surface on the SIS with retention of less cellular material. Even with PAA treatment, the SIS retained about 70-80% of its normal tensile strength. The cells formed uniform multiple layers on the lamina propria of the SIS matrix and penetrated deeper into the porous matrix during dynamic culture. In contrast, cells layered only on the top of matrix and showed minimal cell-matrix infiltration when cultured on a non-porous matrix or under static culture conditions. Induced USC expressed UC markers (AE1/AE3 and uroplakin-Ia) or SMC markers (alpha-smooth muscle actin, desmin, and myosin) in vitro and in vivo, similar to those formed when bladder urothelial and smooth muscle cells were seeded on SIS constructs.

Discussion and Conclusions

The dynamic culture system promoted 3D cell ingrowth into the highly porous collagen matrix of SIS. USC were capable of differentiation into UC and SMC, which then form the normal, layered structure of urinary tract tissue in vivo. These cells may be potentially useful in cell-based tissue engineering for urethral reconstruction or other soft tissue repair.

Disclosures: The authors have nothing to disclose.