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Differential affinity of vitronectin versus collagen for synthetic biodegradable scaffolds for urethroplastic applications

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ABSTRACT

Cell-seeded synthetic polymer scaffolds constitute an emerging technology for urethroplastic applications. The study goal was to identify urethral proteins appropriate for cell attachment and optimize their adsorption onto two types of scaffolds: porous poly(ester urethane) with a poly(caprolactone) soft segment (PEU-PCL) and poly-(96% L/4% D)-lactic acid (P96L/4DLA). Specimens from eight men undergoing urethral reconstruction for stricture diseases were subjected to immunohistochemical analysis. Type I collagen, type IV collagen and vitronectin were detected at the interface between the epithelium and its basement membrane. Electrophoresis confirmed that polypeptide chains in the starting material were also present in fractions eluted from adsorbed scaffolds. Over a 4 week incubation assay, only vitronectin exhibited 100% retention levels for all scaffolds. The saturation point for each protein on each scaffold type was determined by titration and ELISA. The collective evidence indicates the concept that vitronectin > type IV collagen > type I collagen are preferred adsorption proteins for PEU-PCL and P96L/4DLA.

1. Introduction

Various clinical conditions affect the function of the urethra in individuals of all ages. Urethral s*tricture disease is a worldwide problem caused by stenosis of the epithelial duct in response to inflammation, disease, surgery or trauma.* Urethral stricture impairs the ability to void to completion. Hypospadias occurs when portions of the urethra are absent or incorrectly formed. Surgical means to treat and improve these conditions exist; however, durable successful repair remains low in a subset of these conditions. For example, a 15 year follow-up study of substitution urethroplasty found a re-stricture rate of 58% and the overall complication rate to be 33% [1].

The epithelial lining of the urethra differs between men and women. In the female, the 4–5 cm long lining consists of stratified squamous epithelium, which becomes transitional (urothelium) at the bladder neck. In the male, the histological anatomies of the ~20 cm long lining permits the urethra to be classified into the following 3 regions (although 6 regions have been described) [2]: (i) prostatic (urothelial), (ii) membranous (pseudostratified columnar) and (iii) penile (pseudostratified columnar proximally and stratified squamous distally). The penile urethra has been shown to be devoid of specialized uroplakin proteins [3], which form asymmetric unit membranes in the urothelium of the renal pelvis, ureters, bladder and prostatic urethra. The urethra is a tissue that exhibits extensibility and compliance, is located within the spongy erectile tissue of the corpus spongiosum, and thus closely interacts with vascular spaces and blood compartments.

The selection of the appropriate urethral reconstruction technique is dependent on patient parameters such as anatomy (e.g. stricture, hypospadias, prior surgeries), health (e.g. infection, tumors), patient preferences and post-operative goals. Techniques to replace the resected urethra can be divided into partial substitution methods based on urethroplasty for stricture disease and methods for certain etiologies in which a whole new urethral tube must be generated. Because the urethra is contained within the penis, the importance of restoring normal voiding patterns and sexual functions become paramount. Since reconstruction efforts to address congenital penile malformations in infants, children, adolescents can dramatically affect the quality of life when the

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patient ages into an adult, the consequences of surgery can impart significant psychological and physical effects. Hence the need for improved reconstructive techniques becomes obvious in a modern urological practice. Given that the anterior urethra is intimately attached to the corpora cavernosa along its length, reconstructed tissue would be required to retain its expansile properties; the scaffolds need to dissolve completely so that no foreign material remains in the lumen as a nidus for stone formation; and that the material is resistant to extremely rapid degradation in the urinary milieu.

A replacement urethra, engineered *in vitro* from autologous cells, may hold potential to improve success rates and decrease complications in the surgical repair of urological organs [4–6]. Several methods can be used to tissue engineer replacement urological organs and tissue grafts. First, either non-seeded or seeded cell techniques can be used. Non-seeded tissue grafts rely on the ingrowth of neighboring cells into the scaffolds, whereas cell-seeded grafts have been infused with a culture of the appropriate cells from the patient. Non-seeded grafts may be limited to lengths of about 1 cm because they rely on the presence of healthy tissue neighboring the graft [7]. Seeded grafts, in contrast, may be useful for larger tissue defects because they do not rely as heavily on the ingrowth of cells from adjacent tissue [8].

A diversity of scaffold types exist for use with cell-seeded tissue engineering [8], including biological scaffolds such as decellularized porcine small intestine submucosa, and synthetic biodegradable biomaterials such as poly(lactic acid) and poly(ester urethane) (PEU) with poly(caprolactone) (PCL) as a soft segment and lysine units in the hard segment. Some studies have found biological scaffolds to be promising because they contain cell signaling molecules necessary for the differentiation of tissues [9,10]. However, although these materials contain important signaling molecules, they do not have the batch-to-batch consistency that synthetic polymer scaffolds can have. Synthetic materials offer the benefit of batch-to-batch reproducibility as well as protein signaling and the ability to incorporate nanoscale features [11]. Also, synthetic biodegradable scaffolds have an advantage over biological scaffolds because of their inherent ability to resist immunological rejection.

Synthetic biodegradable scaffolds can be made from various polymers with widely ranging morphologies and structural properties. Polyurethanes have a long history of use in medical applications and the mechanical properties can be tuned to specific applications [12]. Recently, a growing interest in the use of specialized poly(ester urethane)s for tissue engineering applications has emerged due to their well characterized degradation products, which are cleared by the renal system [12]. Such degradation products are formed by hydrolysis of ester linkages to form α-hydroxy acids and urethane/urea fragments, of which the latter can undergo further hydrolysis into lysine residues. Numerous studies have reported that the degradation products of PEUs are non-cytotoxic, as reviewed in [12]. Another important characteristic of porous PEU scaffolds is their excellent mechanical properties that can be tunable for the specific application by changing the molar ratio of the initial components. PEU scaffolds possess elastomeric properties that are of great value for applications where elasticity and strength are required. PEUs are capable of being molded into tubes, a highly desirable property when a replacement urethra is required. The hydrolytically and enzymatically-sensitive aliphatic ester linkages of PCL can be degraded [13] and its degradation product, 6-hydroxy hexanoic acid, can be transformed by microsomal ω-oxidation to adipic acid prior to metabolic clearance [14]. There is a wide range of urethral properties, depending on the location in the urethra, and the sex of the individual. The possibility to match the mechanical properties of the urethra to the scaffold material will be an essential property of a tissue engineering scaffold. It is therefore possible to tune mechanical behavior from a hard-tough polymer (PLA) to a highly elastomeric structure (PEU).

Another promising synthetic biomaterial is poly(DL-lactic acid), for example P96L/4DLA, that is available commercially from Scaffdex (Tampere, Finland) as a film or as a braided tubular stent. These polymers degrade with time but retain structural rigidity, do not have toxic byproducts when degraded, and have been shown to perform adequately when implanted in humans, rabbits, and mice [15]. They have previously been used as structural stents in dog ureters [15,16].

Previous research has shown that the untreated surface of biomaterials such as PEU-PCL or P96L/4DLA may not be conducive to cellular adhesion [8,17]. We have previously demonstrated that adsorbing scaffold surfaces with extracellular matrix proteins can improve cellular attachment, result in increased DNA synthesis, and increase proliferation of urothelial cells [6]. Other studies have shown that the adsorption of type I collagen or vitronectin on polystyrene culture dishes greatly increased the ability of weakly adhered 293 cells to remain adhered to the polystyrene surface when exposed to 0.10 Pa shear stress [18].

While collagen has been described in the urethra, the literature is devoid of reports that describe the detection and function of vitronectin in the urethral epithelium. Vitronectin is synthesized and secreted into the bloodstream by the liver where it circulates as an inactive monomer prior to extravasation into subendothelial spaces. When plated on a vitronectin-containing substratum, many cell types, including epithelial and smooth muscle cells, are capable of interacting with $\alpha_v\beta_3$ integrin receptors present on cell surfaces [19–21]. Such interactions lead to the 3 components of cell-matrix adhesion: attachment, spreading and the formation of focal adhesions. In general, vitronectin is known to display the following 3 types of activities: (i) interaction with integrin receptors to gain adherence, (ii) interaction with growth factors to gain proliferation, and (iii) interaction with the urokinase-type plasminogen activator receptor (uPAR) to protect against the membrane-damaging effect of the terminal cytolytic complement pathway. An innovative concept in the field of urethral tissue engineering can therefore be based on a combination of (i) synthetic biomaterials such as PEU-PCL and P96L/4DLA, (ii) autologous urethral cells and (iii) autologous vitronectin isolated from a patient's blood. A better understanding of the adsorption properties of vitronectin is therefore clearly needed for tissue engineering applications.

2. Materials and methods

2.1. Chemicals and reagents used

Bovine serum albumin (fraction V) (catalog # 001-000-162), rabbit anti-mouse conjugated to Cy-2 (catalog # 315-225-045) and horseradish peroxidase-conjugated goat anti-mouse IgG (catalog # 115-035-146) were purchased from Jackson ImmunoResearch (West Grove, PA). Brilliant Blue R-250 (catalog # B-0149), sulfuric acid (catalog # 258105), type IV collagen (Human Placenta, catalog #C5533), xylene substitute (catalog # A5597), streptavadin-peroxidase (catalog # S-2438) and phosphate buffered saline with Tween-20 (PBS-T), pH 7.4 (catalog # P-3563) were purchased from Sigma–Aldrich (Saint Louis, MO). Precast NuPAGE 4%–12% bis-tris polyacrylamide electrophoretic gels (catalog # NP0335BOX), NuPAGE MOPS running buffer (catalog # NP0001) and NuPAGE LDS sample buffer (catalog # NP0007) were purchased from Invitrogen (Carlsbad, CA). 3,3′,5,5′-tetramethylbenzidine (TMB) substrate reagent set (catalog # 555214) was purchased from BD Biosciences (San Jose, California). 96-well plates (catalog # 3585) were purchased from Corning Life Sciences (Lowell, MA). Mouse anti-type IV collagen IgG (catalog #M3F7) was purchased from the University of Iowa Hybridoma Bank (Iowa City, IA). Mouse anti-human type I collagen IgG (catalog # ab6308) and mouse anti-human vitronectin IgG (catalog # ab13413) were purchased from Abcam (Cambridge, MA). Superfrost Plus microscope slides (catalog # 12-550-15), Citric acid (catalog # A104), 30% hydrogen peroxide (catalog # BP2633), Methanol (catalog # A412), glacial acetic acid (catalog # A38) and Chloroform (catalog #C298) were obtained from Fisher Scientific (Waltham, MA). Ethyl Alcohol, 200 proof USP (catalog # 111100200CSGL) was