

IDEAL STABILIZATION OF IN VITRO ENGINEERED UROTHELIUM WITH FIBRIN GLUE

Hypothesis / aims of study

Despite surgical techniques in reconstructive urology have advanced considerably in recent years, an equal substitute for urothelium is eligible (1). In vitro engineered autologous urothelium is a promising surrogate in reconstructive urology involving urethral reconstruction (e. g., repair of urethral strictures, fistula, or hypospadias) and bladder reconstruction (e. g., bladder exstrophy). For in vitro stratified urothelial cell-matrix implants appropriate matrices are a continued challenge (2). Applicable matrices must be biocompatible and degradable in vivo. To overcome these problems, in vivo engineering of stratified urothelium without matrices or scaffolds is an alternative technique. However, matrix-free urothelium is fragile and thus has to be stabilized for use in reconstructive surgery (3). The ideal stabilization factor should not influence the viability and protect the engineered constructs temporarily from urine. In addition, it should not promote inflammation and fibrosis, and should be degradable in vivo. The aims of the study were to 1) prove the viability of fibrin glue-sprayed human urothelial cells, 2) stabilize in vitro engineered urothelium using fibrin glue, and 3) to investigate the outcome of tissue-engineered human urothelial transplants stabilized with fibrin glue in a pilot nude rat model.

Study design, materials and methods

The influence of fibrin glue on the viability of proliferating and confluent monolayered human urothelial cell cultures was analyzed with the metabolic WST-1 assay. Seven in vitro engineered urothelia established from three different primary human urothelial cell lines were enzymatically detached, then sprayed with fibrin glue, and investigated for mechanical stability. For verifying the outcome in vivo, engineered urothelia were sprayed with fibrin glue and sutured on the musculus rectus abdominis of athymic rats. For in vivo tracking, human urothelial cells have been labeled with red fluorescent PKH26 cell linker. Transplants were examined histologically and immunologically for epithelial pancytokeratin (AE1/AE3) after seven days.

Results

Viability of fibrin glue-sprayed human urothelial cell cultures both in the proliferative and confluent phase reached up to 62% and 89%, respectively, of the control group at day seven. Engineered urothelia sprayed with fibrin glue demonstrated a good mechanical stability compared to unsprayed urothelial constructs. The urothelia stabilized with fibrin glue were well handable with surgical instruments. In performed cryosections of engineered urothelia at day seven after transplantation a good integration in the target tissue and the epithelial phenotype could be demonstrated. Fibrin glue was nearly degraded. There was no sign of any inflammatory reaction.

Interpretation of results

Clinical application of in vitro engineered urothelium without matrices or scaffolds requires stabilization factors due to its mechanical instability. Spraying with fibrin glue enhanced the mechanical stability of engineered urothelia so that they could be well manipulated with surgical instruments. The impact of fibrin glue on the vitality of human urothelial cells was considerable low. The pilot animal model demonstrated survival and integration in the host tissue of engineered urothelium after transplantation. These findings are encouraging and suggest fibrin glue as a biocompatible stabilizer for in vitro engineered urothelium.

Concluding message

In vitro engineered autologous urothelium stabilized with sprayed fibrin glue is a promising substitute for sustained regeneration of urothelium in reconstructive surgery of the lower urinary tract. It might expand the toolbox in reconstructive urology. Direct application of engineered urothelium could replace flaps in open urethral surgery and might be used in endoscopic urethroplasty.

References

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3. Nagele U, Maurer S, Feil G, Bock C, Krug J, Sievert K-D, Stenzl A: In vitro investigations of tissue-engineered multilayered urothelium established from bladder washings. *Eur Urol* 2008; 54(6): 1414-22

<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	Ethics Committee of the University of Tübingen