LIVING SKIN EQUIVALENT AS A UNIVERSAL TOOL FOR CLOSING FULL-THICKNESS EPITHELIAL-STROMAL SKIN, URETHRAL AND UPPER RESPIRATORY TRACT INJURIES

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Introduction

Despite advancements in bioengineered therapies and reconstructive surgery effective closure full-thickness epithelial-stromal injuries still is a serious clinical problem. The possibility of living skin equivalent (LSE) – 3D collagen-based structure, containing keratinocytes and mesenchymal cells, to stimulate wound healing of skin, urethra and trachea was investigated.

Methods

Three animal models were used in this work: murine model of full-thickness excisional splinted wound, rabbit model of circular defect of the urethral epithelium, and rabbit model of full-thickness trachea defect. LSEs were fabricated from mesenchymal cells-populated collagen gel followed by seeding of cultured keratinocytes on the surface of it. Histological and immunohistochemical methods were used to study the regenerative abilities of LSE.

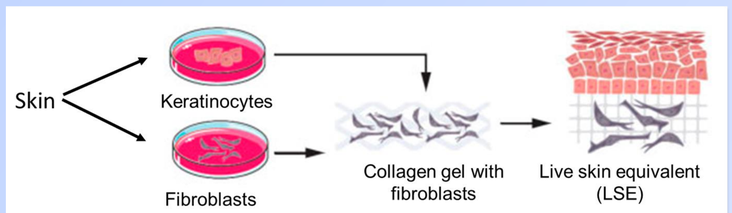

Fig.1. Scheme of LSE preparation

Results

LSE as a wound dressing

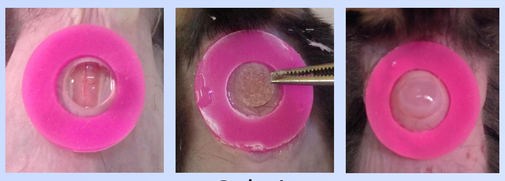

Fig.2. A murine model of the full-thickness excisional splinting wounds

It was shown that LSE applied for full-thickness skin injury promoted immediate filling of wound bed and provided simultaneous reorganization of dermal component into highly-vascularized granulation-like tissue and rapid epithelialization, thus improving the quality of healing. Inflammation was delayed and less pronounced.

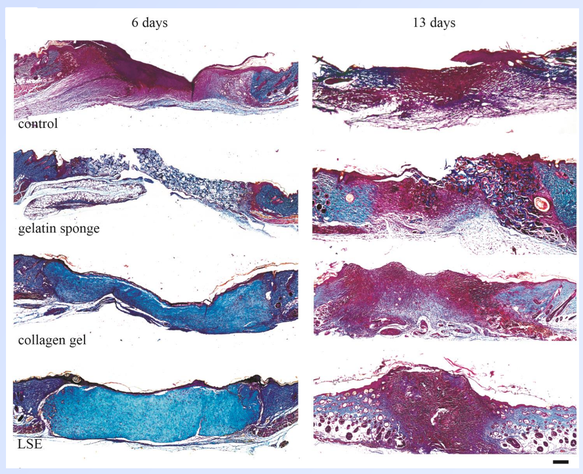

Fig.3. Picro Mallory trichrome staining demonstrates collagen content and matrix remodeling. Scale bar - 200 µm.

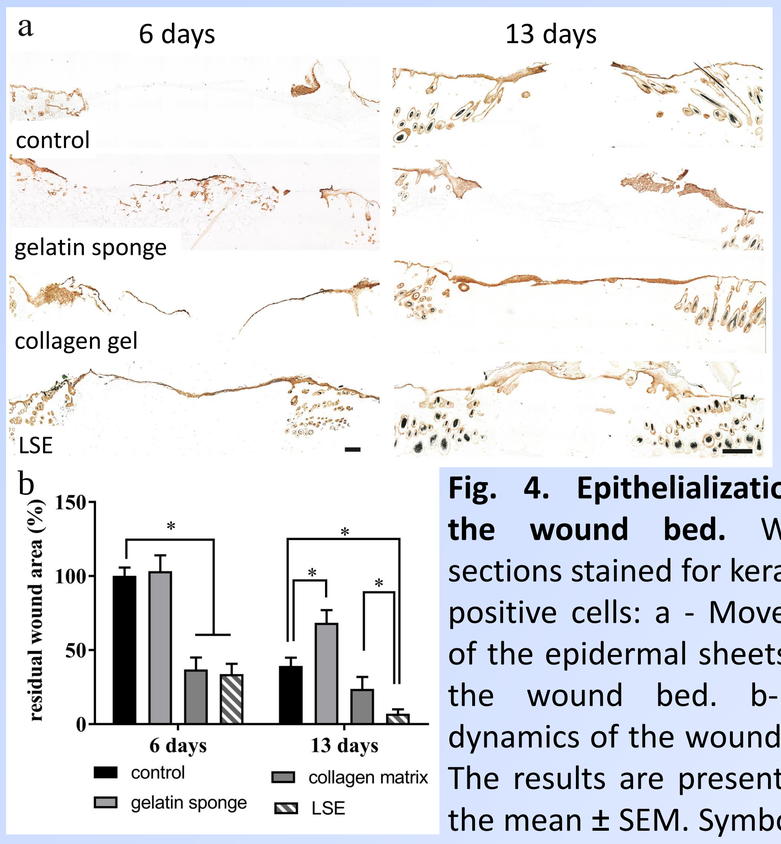

Fig. 4. Epithelialization of the wound bed. Wound sections stained for keratin 5-positive cells: a - Movement of the epidermal sheets over the wound bed. b- The dynamics of the wound area. The results are presented as the mean ± SEM. Symbol: * = p<0.05.

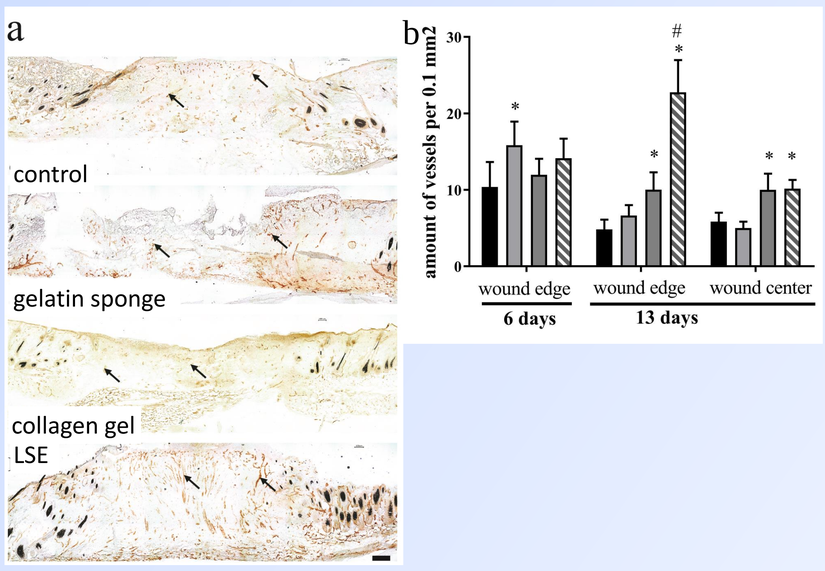

Fig.5. Angiogenesis of the wound: (a) immunostaining for CD31 (exemplified by arrows), (b) diagram of vessel count. Scale bars in (a) are 200 µm. The results in (b) are presented as the mean ± SEM, Symbols: * p<0.05 relative to the control, # p<0.05 relative to collagen gel.

Urethra reconstruction with LSE

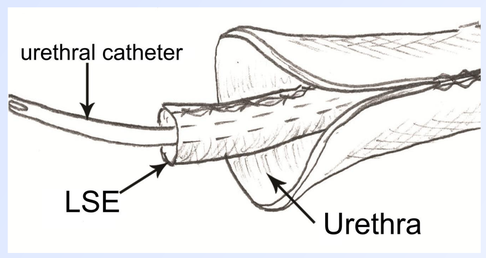

Fig.6. Scheme of the LSE transplantation into de-epithelialized urethra

Histological analysis of urethra revealed the complete closure of the defect by squamous epithelium 21 days after LSE transplantation, the epithelium of neourethra corresponded to the normal after 90 days. EGFP transfected donor cells co-expressed urothelial markers (keratin 7, uroplakin) at all timepoints of experiment.

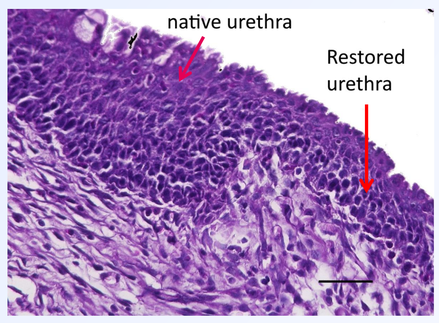

Fig.7. Section of restored rabbit urethra 45 days after LSE transplantation. The site of anastomosis of the native urethra and the restored urethra . Hematoxylin and eosin staining, scale bar – 50 µm

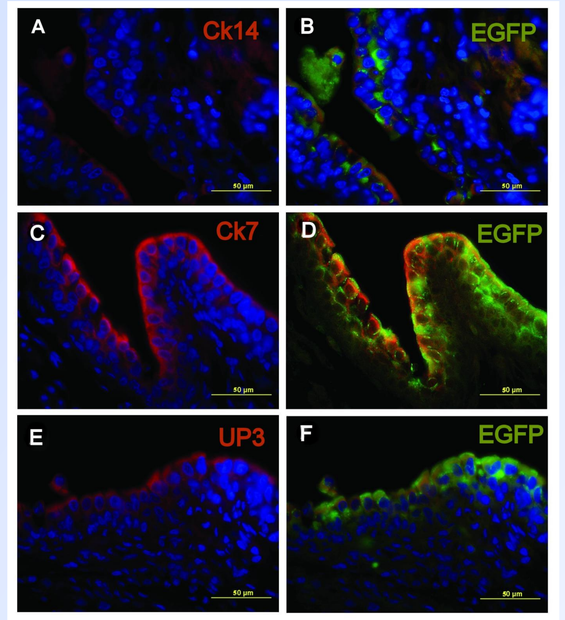

Fig. 8. Section of rabbit urethra 45 days postoperatively. Restored urothelium, immunofluorescent detection of urothelial markers. Colocalization of genetic tag EGFP (at B, D and F - green) and the marker of the epidermis – cytokeratin 14 (CK14, red for A and B), the markers of the urothelium – cytokeratin 7 (CK7, red on C and D), and uroplakin 3 (UP3, red on E and F). Nuclear staining - DAPI (blue).

Upper respiratory tract reconstruction with LSE

Histological analysis of trachea revealed the closure of the defect with single-row epithelium 14 days after reconstruction with LSE, while control defect was not epithelized. Defects were epithelized in control and LSE-treated animals after 30 days, but control group showed strictures and inverted scars formation at the site of defect.

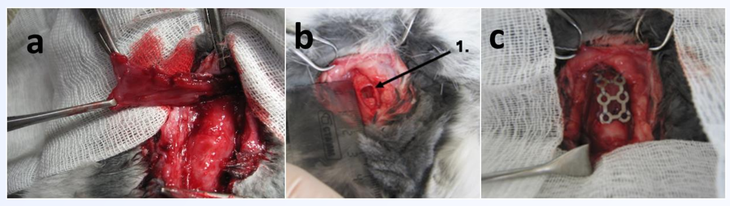

Fig. 9. LSE was implanted on the muscle for 2 weeks, then an epithelial-muscle flap (a) was formed, which was used for the trachea defect (b) reconstruction, titanium mesh was used as tracheal support (c)

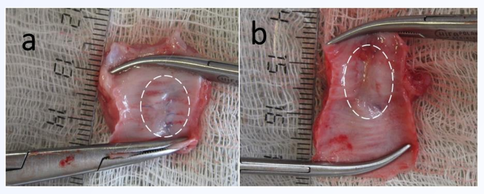

Fig. 10. Photos of trachea 30 days after reconstruction with LSE (a) – without scar formation, and control defect (b) with a scar

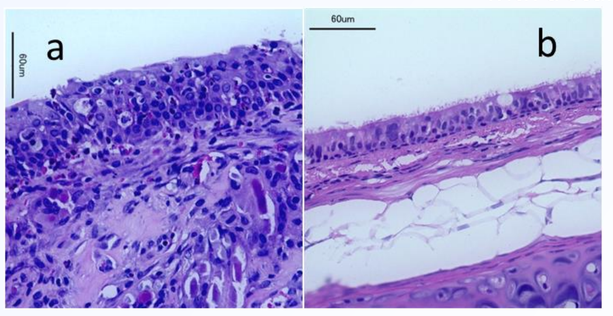

Fig.11. Section of restored rabbit trachea 15 days after operation (a), (b)-native trachea. Hematoxylin and eosin staining.

Conclusion

LSE can temporarily integrate into the recipient tissues and to stimulate skin wound healing and epithelialization of the trachea defects without the scar formation. LSE, composed of autologous keratinocytes, can embed into the structure of the urethra upon the transplantation into the urethra defect, restoring its integrity and functionality.

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