Original Article

The Sheep's Urinary Bladder Matrix as a Potent Biological Materials Resource -an Ultrastructural Study

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ABSTRACT

Background and Objectives: Biological scaffold resources composed of extracellular matrix (ECM) have been shown to make easy the practical remodeling of various tissues in both animal and human studies. The goal of current study was to make sheet form of ECM from sheep's urinary bladder.

Materials and Methods: ECM was extracted from Sheep's urinary bladder according to standard method. Scanning electron microscopy (SEM) was applied in order to analyze the ultrastructure of the extracted matrix.

Results: Matrix was formed by irregular and fiber like particles.

Conclusion: Sheep's urinary bladder matrix may be used as an accessible and suitable source of ECM extraction.

Keywords: Extracellular matrix, Sheep, Scanning Electron Microscopy, Urinary Bladder

Introduction

The extracellular matrix (ECM) is the extracellular component of animal tissue that generally provides structural support to the cells and has various important growth factors. Biological scaffold resources composed of ECM have been shown to make easy the constructive remodeling of numerous different tissues in both animal and human studies (1).

The ECM is obviously biocompatible since host cells generate their own matrix. The ECM also provides a supportive medium for lymphatics, nerves and blood vessels and for the diffusion of nutrients from the blood to the contiguous cells. In other words, the ECM possesses all of the characteristics of the ideal tissue biomaterial or engineered scaffold (2).

Bladder extracellular matrix consists of basement membrane and other extracellular matrix

components. Basement membrane characteristics are ideal for epithelialization (3). There are a lot of diverse collagen types and growth factor, glycoprotein and disinfectants peptides (4). In a recent study on diabetic patients, the healing effect of this urinary bladder matrix was proved. This study shows that pig's bladder is a good source for extraction (5). ECM scaffold is effective in thoracic wall reconstitution (6). The significant role of ECM is proved in wound healing and regenerative purposes provoked us to examine a new and available method for ECM extraction in Iran.

In the present study, for the first time, we extracted the ECM from sheep's urinary bladder (as a waste material) and confirmed its characters with Scanning Electron Microscopy (SEM).

Materials and Methods

Sheep urinary bladders were harvested from Kerman market. Residual external connective tissues, including adipose tissue, were trimmed and all residual urine was removed by repeated washes with tap water. The epithelial layer was removed by soaking of the material in 1 N normal saline. The serose layer, muscularis layer, submucosal layer were mechanically separated from the bladder tissue. The remaining basement membrane of the epithelium and the subjacent lamina propria, collectively termed UBM, were then decellularized and sterilized by immersion in 0.1% (v/v) peracetic acid (s), 4% (v/v) ethylic alcohol, and 96% (v/v) deionized water for 2 h. The UBM-ECM material was then washed twice for 15 min with phosphate-buffered saline (PBS) (6).

Lyophilization

Following the preparation of the ECM, it was lyophilized using an FTS Systems Bulk Freeze Dryer Model 854. The extracted matrix was cut into small pieces and stored in air-tight sealed packages. The samples were terminally sterilized by e-beam irradiation at 22 kGy (Titan Scan Technologies, OH). The lyophilized matrix was stored at room temperature (20–24°C).
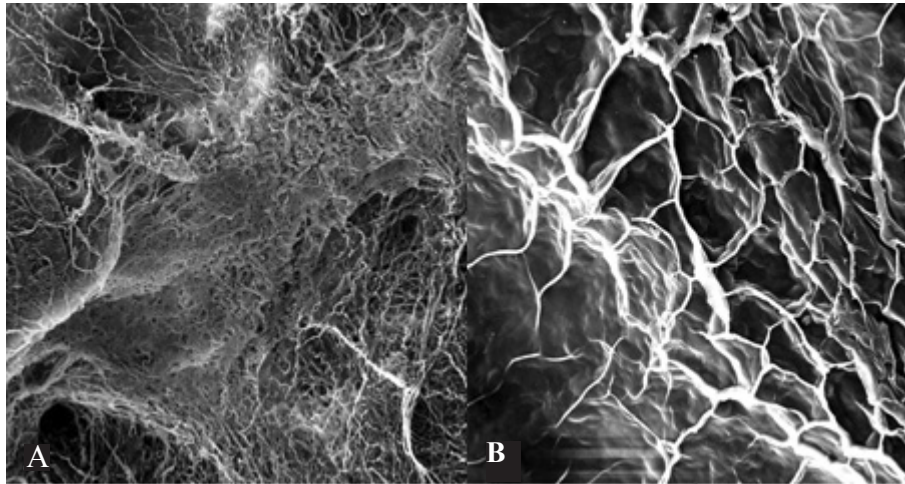
Scanning Electron Microscopy

The ultrastructure of the ECM components was analyzed using SEM (VGA TS5130MM). A thin layer of the extracted matrix was mounted on adhesive metallic tape. It was sputter coated with 3.5 nm of gold. The samples were viewed at magnifications from 250 to 6700.

Results

Lyophilized extracted matrix after processing, is visible in Fig. 1, which is a white material width 0.12±0.01mm. The ultrastructure of extracted matrix was evaluated with SEM. The particles were irregularly shaped and could be defined generally as fiber-like (Fig. 2). Examination of the specimens at higher magnification showed that collagen fibers are associated with small particles on the order of 1 μm in diameter.

Fig. 1:   Lyophilized sheep's ECM sheet after processing

Fig. 2: Scanning electron micrograph of sheep's ECM components. Figure presented fiber like particles in the ECM. Magnification is ×250 in A and ×1000 in B

Discussion

The goal of current study was to make sheet form of ECM from sheep's urinary bladder. In previous studies ECM was extracted from porcine bladder in USA that is not available in Iran and this is the first study of extracting ECM in Iran. This source have economic benefits because this making gold from rubbish.

SEM is suitable to evaluate the ultrastructure and particle size distribution for the material in the study. The irregular shape and size of the particles is likely due to the fibrous nature of this mostly collagenous material. Individual fibers are visible both alone and associated with other particles. This finding is in agreement with Gilbert's study (7).

The ECM is derived from a variety of tissues, such as small intestinal submucosa (SIS) (8), urinary bladder (7, 9), liver (10) and etc. ECM scaffolds contain the structural and functional molecules secreted by the inhabitant cells of each organ from which they are prepared. Thus, the specific composition and allocation of the ECM constituents will be different depending on the tissue source. The ECM scaffold derived from porcine small intestinal submucosa (SIS–ECM) is the biological scaffold material that has been most expansively characterized, and therefore will be used as a prototypical ECM scaffold. SIS–

ECM scaffold is composed of greater than 90% collagen. The greater part of the collagen is type I, with minor amounts of collagen types (Col) III, IV, V and VI also present (11). Porcine urinary bladder matrix (UBM–ECM) also contains the same collagen types as SIS–ECM, with greater amounts of Col III being present, as well as Col VII. Col VII is an important component of the epithelial basement membrane that distinguishes this particular ECM scaffold from most other ECM scaffold materials (12).

In conformity with this finding, our ultrastructural study showed that the extracted particles were irregularly shaped and could be defined generally as collagen fibers. On the other hand, various growth factors are also present in ECM, including transforming growth factor-β (13, 14), basic fibroblast growth factor (b- FGF) (15) and vascular endothelial growth factor (VEGF) (16). Numerous of these growth factors have been shown to retain their bioactivity even after terminal sterilization and long-term storage (13, 15).

Conclusion

Extraction of ECM from sheep's urinary bladder is achievable and the extracted matrix may be usable for regeneration purposes. Additional study is needed to investigate the mammalian tissue response to the extracted ECM, and as well

as to further refine the production techniques for clinical use.

Acknowledgment

References

1. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomaterialia 2009; 5: 1–13.

2. Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007; 28: 3587–93.

3. Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF. The basement membrane component of biologic scaffolds derived from extracellular matrix. Tissue Eng 2006; 12(3): 519-26.

4. Brennan EP, Reing J, Chew D, Myers-Irvin JM, Young EJ, Badylak SF. Antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix. Tissue Eng 2006; 12(10): 2949-55.

5. Kimmel H, Rahn M, Gilbert TW. The clinical effectiveness in wound healing with extracellular matrix derived from porcine urinary bladder matrix: a case series on severe chronic wounds. The Journal of the American College of Certified Wound Specialists 2010; 2(3): 55–9.

6. Gilbert TW, Nieponice A, Spievack AR, Holcomb J, Gilbert S, Badylak SF. Repair of thoracic wall with an extracellular matrix scaffold in a canine model. J Surgical Res 2008; 147(1): 61-7.

7. Gilbert TW, Stolz DB, Biancaniello F, Simmons-Byrdd A, Badylak SF. Production and characterization of ECM powder: implications for tissue engineering applications. Biomaterials 2005; 26: 1431–5.

8. Badylak SF, Lantz GC, Coffey A, Geddes LA. Small intestinal submucosa as a large diameter vascular graft in the dog. J Surg Res 1989; 47(1):74–80.

9. Chen F, Yoo JJ, Atala A. Acellular collagen matrix as a possible ''off the shelf'' biomaterial for urethral repair. Urology 1999; 54(3): 407–10.

10. Lin P, Chan WC, Badylak SF, Bhatia SN. Assessing porcine liver derived biomatrix for hepatic tissue engineering. Tissue Eng 2004; 10(7–8):1046–53.

11. Badylak SF, Tullius R, Kokini K, Shelbourne KD, Klootwyk T, Voytik SL, et al. The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. J Biomed Mater Res 1995; 29(8): 977–85.

12. Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF. The basement membrane component of biologic scaffolds derived from extracellular matrix. Tissue Eng 2006; 12(3): 519–26.

13. McDevitt CA, Wildey GM, Cutrone RM. Transforming growth factor-beta1 in a sterilized tissue derived from the pig small intestine submucosa. J Biomed Mater Res A 2003; 67(2): 637–40.

14. Voytik-Harbin SL, Brightman AO, Kraine MR, Waisner B, Badylak SF. Identification of extractable growth factors from small intestinal submucosa. J Cell Biochem 1997; 67(4): 478–91.

15. Hodde JP, Ernst DM, Hiles MC. An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix. J Wound Care 2005; 14(1): 23–5.

16. Hodde JP, Record RD, Liang HA, Badylak SF. Vascular endothelial growth factor in porcine-derived extracellular matrix. Endothelium 2001; 8(1): 11–24.