Quantification of Topologic characters and Prime Proteins of Esophageal Basement Membrane for Scaffold Design in Tissue Engineering

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Basement membrane (BM) has been investigated widely and applied in the field of bio-synthesized scaffold design in tissue engineering. However, this respect of investigation has seen scarcely in the field of esophageal tissue engineering. This study reports BM's basic topographic characters and quantification of prime proteins involving collagen IV, laminin, entactin, proteoglycans (PG) of porcine esophagus. All results provide references for the design of synthesized scaffolds and protein modification in esophageal tissue-engineering research.

Methods:
Several methods were adopted to strip epithelium from mucosa tissue. For example, ethylene diamine tetraacetic acid (EDTA), sonication, dithiothreitol (DTT), cold sodium chloride (NaCl), mechanical force of glass coverslip, and cold trypsin etc. Assessed experimental results under different conditions, the optimal condition to isolate epithelium from basement membrane was established. After the reaction of cold trypsin at 4□ for 15 h, the BM was isolated from epithelium and exposed integratedly. Hematoxylin and eosin staining (H&E) and immunohistochemical staining verified the effectiveness of epithelium removal and the integrity of BM. The topographic features of the exposed BM were observed under transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The prime proteins, i.e. collagen IV, laminin, entactin and proteoglycans existing in BM were qualitatively and quantitatively analysed by enzyme-linked immune sorbent assay (ELISA) as well as Western-blotting.

Results: Epithelium removal and proteins extraction of porcine esophageal BM were established. After esophagus tissue was treated with 0.4% trypsin/PBS for 15 h at 4 ℃, entire epithelium was stripped off. TEM graphs and immunohistochemistry assay revealed the integrity of exposed BM. It was 3-D images, abundant in pores, and locate between epithelium and connective tissues (Figure 1). Pores were of unequal size, distributed irregularly and composed by interwoven fibers Analyzed by ImageJ software, the thickness of BM was not uniform ranging from 53 to 151 nm with averages of 86 ±15 nm. It was confirmed from H&E staining and immuno-histochemistry assay. The prime proteins, i.e. collagen IV, laminin, entactin and proteoglycans, were qualitatively and quantitatively analysed by enzyme-linked immune sorbent assay (ELISA) (Table 1).It is believable that all these feature data were very important in knowledging the microenvironment of epithelial cells and their behaviors like growth, adhesion and proliferation.

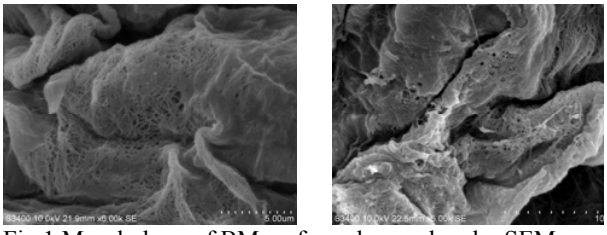

Fig.1 Morphology of BM surface observed under SEM

Table 1 Different kinds of BM proteins using ELISA quantity

	total protein	collagen	laminin	entactin	proteoglycans
	(mg:g wet weight)	(ng:g wet weight)	(μg:g wet weight)	(ng:g wet weight)	(ng:g wet weight)
3.4MNaCl	15.51±3.05	180.75±37.15	275.09±37.96	5.37±0.6	--
3.4MNaCl	9.41±3.19	173.99±12.65	285.97±22.32	5.02±0.7	--
0.5MNaCl	5.45±2.25	167.64±11.68	339.44±31.61	5.2±0.8	--
2.0MGu·HCl	12.79±2.70	144.62±62.41	201.38±28.71	2.38±0.25	35.95±4.68
4.0MGu·HCl	11.08±1.02	94.85±13.51	94.82 ±21.38	3.13±0.41	97.75±6.97
autoclave	4.88±0.70	117.73±8.67	264.62 ±25.66	4.46±0.76	--
total quantity	59.11±6.63	908.91±58.37	1461.34±60.03	25.66±1.29	119.82±25.92
without muscle	301.24±40.25	4246.58±394.39	6853.63±809.59	132.76±25.91	483.52±134.22

Conclusions: This study reports BM's basic topographic characters and quantification of prime proteins involving collagen IV, laminin, entactin, proteoglycans (PG) of porcine esophagus. Several methods were adopted to strip epithelium from mucosa tissue. The optimal protocal, i.e. cold trypsin, was established. Hematoxylin and eosin staining (H&E) and immunohistochemical staining verified the effectiveness of epithelium removal and the integrity of BM. It displayed a rugged surface and 3-D topography consisting of pores and fibers with sub-100 nm range. The prime proteins existing in BM were qualitatively and quantitatively analysed by enzyme-linked immune sorbent assay (ELISA) as well as Western-blotting. All these results provide references for the design of synthesized scaffolds and protein modification in esophageal tissue-engineering research.

Acknowledge:
The authors gratefully acknowledged the financial supports from national science foundation (30870645) and provincial outstanding youth team foundation (R2101166).