COMPARATIVE EVALUATION OF PORCINE AND BUFFALO SMALL INTESTINAL SUBMUCOSA FOR THE DEVELOPMENT OF EXTRACELLUALR MATRIX SCAFFOLDS*

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Abstract

Small intestinal submucosa (SIS) from 6-8 months old Large White Yorkshire (LWY) pigs and 18-24 months old buffaloes were evaluated for the development of extracellular matrix scaffolds. The thickness of SIS from buffalo and LWY pigs did not differ significantly. Buffalo SIS had significantly higherfat and total collagen contents, and was more resistant to collagenase degradation than that from LWY pigs, but showed a higher content of acid soluble collagen. Buffalo SIS could be a better raw material for scaffold preparation as it is a widely available low-cost by-product. However, the properties of the scaffold may alter with subsequent treatments like defatting, decellularization, cross linking and sterilization.

Key words: *Extracellular matrix scaffolds, small intestinal submucosa, pig, buffalo*

Extra cellular matrix (ECM) is the tissue from which the resident cells have been removed with minimum alterations to the other components of the matrix. Biological scaffolds materials composed of ECM have been developed to facilitate the constructive remodelling of many different tissues in preclinical animal studies and human clinical applications. Small intestinal submucosa (SIS) is an easily available, largely wasted abattoir by-product which can be used for the preparation of ECM scaffolds. The objective of this study was to compare various physico-biochemical properties of buffalo and porcine SIS to assess their suitability for scaffold preparation.

Materials and Methods

Six Large White Yorkshire pigs (LWY) of 6-8 moths age and six male buffaloes of 18-24 months were randomly selected and brought from farms of Kerala Veterinary and Animal Sciences University (KVASU) and slaughtered hygienically at Meat Technology Unit, KVASU, Mannuthy. Small intestinal segments from the jejunum portion of the slaughtered animals were harvested and manually processed to yield the SIS. The raw porcine and buffalo SIS were compared with respect to the following parameters.

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1. Thickness
2. Chemical composition, viz. moisture, fat and total protein (AOAC, 2010 and Starcher, 2001)
3. Total collagen content (Stegman and Stadler, 1967)
4. Neutral salt soluble and acid soluble collagen (Reddy *et al*., 2002)
5. Resistance to *in vitro* enzymatic degradation(Paul and Bailey, 2003)

Data obtained were compiled and analysed statistically as per the methods of Snedecor and Cochran (1994) using the SPSS software package (Version 21).

Results and Discussion

The small intestinal segments from the jejunum portion of buffaloes and LWY pigs were collected, SIS prepared and assessed for various quality parameters.

1. Thickness

Thickness of SIS from LWY pigs and buffaloes is presented in Table 1. The total thickness of small intestinal submucosa was not significantly different between the two groups of animals. The results are in agreement with already reported values for thickness of SIS segments (Andree *et al.,* 2013).

2. Chemical composition

Chemical composition of SIS from LWY pigs and buffaloes is presented in Table 1. Porcine SIS had significantly lower fat content on a dry fat-free basis.

3. Total collagen content

Total collagen content of SIS from LWY pigs and buffaloes is presented in Table 2. Buffalo SIS has significantly ($p<0.05$) higher per cent total collagen content (85.54±1.34) when compared to SIS from LWY pigs (67.15±1.02).

Table1. Thickness and chemical composition of Small Intestinal Submucosa from LWY pig and Buffalo

Animal group	Thickness (µm)	Moisture (%)	Fat (%)	Total Protein (% dry fat free basis)
LWY	84.5±15	82.04±1.46^{b}	2.31±1.6^{a}	90.73±6.36
Buffalo	77.5±38	78.01±0.51^{a}	9.43±2.29^{b}	93.78±1.63

Means bearing different superscripts in the column differ significantly (P<0.05) LWY-Large White Yorkshire pig

Table 2. Collagen characteristics of Small Intestinal Submucosa from LWY pig and Buffalo

Animal group	Total Collagen (% dry fat free basis)	Neutral salt soluble collagen	Acid soluble collagen
		(% of total collagen)	
LWY	67.15±1.02^{a}	1.352±0.57	0.221±0.02^{a}
Buffalo	85.54±1.34^{b}	0.457±0.06	0.61±0.04^{b}

Means bearing different superscripts in the column differ significantly (P<0.05) LWY-Large White Yorkshire pig

Table 3. Weight loss of Small Intestinal Submucosa from LWY pig and Buffalo after enzymatic degradation

Animal Group	Percent weight loss after incubation for		
	24 hrs	48 hrs	72 hrs
LWY pig	50.21±0.98^{b}	61.80±4.41^{b}	89.50±2.63^{b}
Buffalo	36.45±3.32^{a}	46.33±2.61^{a}	67.49± 4.72^{a}

Means bearing different superscripts in the column differ significantly (P<0.05) LWY-Large White Yorkshire pig

It is already reported that the non-water portion of SIS contained approximately 90 per cent protein, and this protein is predominantly made up of collagen (Etris *et al*., 2002).

4. Neutral salt soluble and acid soluble collagen

Neutral salt soluble collagen (NSC) and acid soluble collagen (ASC) contents of SIS from LWY pigs and buffaloes are presented in Table 2. The buffalo SIS had a significantly higher ($P<0.05$) ASC content, whereas the NSC contents were not significantly different between the groups.

5. Resistance to invitro enzymatic degradation

Resistance to enzymatic degradation of LWYpigand buffalo SIS, measured as per cent weight loss after incubation with collagenase, is presented in Table 3. It was observed that buffalo SIS was significantly more ($P< 0.05$) resistant to enzymatic degradation when compared to porcine SIS which might be due to the crosslinking that had happened in the SIS of buffaloes.

Buffalo SIS had significantly higher fat and total collagen contents, and was more resistant to collagenase degradation than that from LWY pigs. However, buffalo SIS showed a higher content of acid soluble collagen. The results suggest that buffalo SIS may be a better source material for ECM scaffold preparation than SIS from LWY pigs. But the final properties of the material may be substantially altered by subsequent processing steps in the scaffold development including defatting, decellularization, crosslinking and sterilization.

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