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In vitro biomechanical property of porcine Acellular diaphragm matrix (ADM) used for surgical reconstruction in abdominal defects

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Abstract
The biological materials used in reconstructive surgery harvested from other animal required chemical or enzymatic treatment to remove the cellular components in order to minimize its antigenicity. In the present study, sodium deoxycholate treated decellularized Porcine Acellular Diaphragm Matrix (ADM) tested for its biomechanical properties compare to native fresh porcine diaphragm in an Instrons Universal Tensile Machine (UTM). Four different sizes of ADM and Fresh Diaphragm (5, 10,20 and 40 mm) tested in the machine keeping constant gauze length 50mm. Prepared Porcine Acellular Diaphragm Matrix (ADM) retained sufficiently high tensile strength (93.94%) and elongation percentage (98.44%) when compared with fresh unprocessed diaphragm.

Keywords: Tensile strength, tissue elongation acellular diaphragm matrix, porcine

Introduction

The biomechanical properties of tissue materials are provided describing the specimen by maximum force, breaking strain and stiffness (Pott *et al.*, 2012) [5]. The synthetic materials that made of knitted or woven mesh the definition of values related to the cross sectional area are of limited importance as the determination of the thickness of the material is user-dependent and the cross sectional area does not define the amount of load-bearing filament (Cobb *et al.*, 2009) [1]. In-vivo biomechanical property of porcine small intestinal submucosa does not changes with the type of its implant techniques (only, inlay and underlay) in surgical reconstruction of abdominal wall defects in pig (Rainier *et al.*, 2006) [6]. In the present study, sodium deoxycholate salt treated Porcine Acellular Diaphragm Matrix (ADM) was subjected to its in-vitro biomechanical evaluation in compare to unprocessed porcine diaphragm.

Materials and Methods

Preparation of Porcine Acellular Diaphragm Matrix (ADM) carried out as per the method described by Kumar *et al.*, (2015) [3]. Porcine Acellular Diaphragm Matrix (ADM) and Freshly collected diaphragm were thoroughly washed several times in 1X PBS solution and kept in a paraffin dissection tray; then with the help of B.P. Blade and measuring scale required geometry of strip and providing clamping zone at the ends of the strips with narrowed required section were cut in different width viz 5, 10, 20 and 40 mm size. The maximum loading tensile strength, breaking strain, elongation percentage on maximum load and break strain of the ADM and Fresh Diaphragm were measured using Intron Universal Tensile Machine (Model-4444) at Indian Jute Industries Research Association (IJIRA), Export Promotion Industrial Park (EPIP), Amingaon, Guwahati-781031, Assam, India (Fig.1). The tensile strength and tissue elongation of the material were expressed in Kilo-Force and in Percentage respectively. The cut piece of tissue was placed carefully between two holding clamp allowing length between 50 mm Gauge Length (50 mm GL) and pushed the run button to measure the tensile strength and degree of elongation on maximum load and break strain (Fig. 2). The present study was in full compliance with the Institutional Animal Ethics Committee, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

Fig 1

Fig 2

Result and Discussion

The mean Tensile Strength (Kg-Force) and Tissue Elongation (%) in respect of maximum load force and break strain of unprocessed Diaphragm and Acellular Diaphragm Matrix (ADM) with Gauge Length (GL) of 50 mm at different width have depicted in Table 1 The analysis of variance {ANOVA Table 2 (a) & 2 (b)} revealed significant difference of both Tensile Strength (Kg-Force) and Tissue Elongation (%) with tissue width of 5 mm between Unprocessed Porcine Diaphragm and Porcine Acellular Diaphragm Matrix (ADM); however the values differed non-significantly with tissue widths of 10 mm, 20 mm and 40 mm. The Tensile Strength comparison on Maximum Load as well as Break Strain between Unprocessed Porcine Diaphragm with Porcine Acellular Diaphragm Matrix showed an increasing trend toward its original strength with increasing its tissue width. The tissue width of 40 mm of Porcine Acellular Diaphragm Matrix (ADM) on Maximum Load and Break Strain resulted highest 93.94% and 95.91 % of Tensile Strength (Kg-Force) when compared with the Unprocessed Porcine Diaphragm respectively; while the same with 5mm width showed lowest 88.89% and 91.25% respectively.

The Tissue Elongation (%) comparison between Unprocessed Porcine Diaphragm and Porcine Acellular Diaphragm Matrix (ADM) on both Maximum Load and Break Strain measures revealed increasing trend towards the original tissue with increasing the width of the tissue. The tissue width of 40 mm of Porcine Acellular Diaphragm Matrix (ADM) on Maximum Load and Break Strain resulted highest with 98.44% and 98.02 % of Tissue Elongation (%) when compare with the Unprocessed Porcine Diaphragm respectively; while the same with 5mm width showed lowest with 90.07% and 91.09% respectively. In the present investigation, the Tensile Strength (Kg-Force) and Tissue Elongation (%) measured in Instron Universal Tensile Machine (UTM) of Porcine Acellular Diaphragm Matrix (ADM) with both Maximum Load and Break Strain effects resulted in negligible losses of its original mechanical strength as well as its elongation percentage. Nilsen *et al.*, (2016) [4] carried out in-vivo testing of Tensile Strength and Tissue Elongation in Acellular Dermal Matrix (ADM) of two commercial products namely, Flex HD Pliable and Bella Derm following acellulatization protocol and recorded higher strength and stiffness in Bella Derm when compared to Flex HD; however both the product maintained optimum tensile strength to support the abdominal wall as well as losses negligible mechanical strength during processing compared to its native tissues. Eberli *et al.*, (2010) [2] compared in-vivo mechanical properties in different commercial Acellular Dermal Matrices (ADM) namely Flex HD Dermis, Flex HD Acellular Dermis- Thick and Allo Derm of human origin in experimental abdominal wall reconstruction in rabbit model and found optimum tensile strength and tissue elongation to support the abdominal wall.

Table 1: MEAN± SE of Tensile Strength (Kg-Force) and Elengation (%) on maximum load and break strain of Acellular diaphragm matrix (ADM) and fresh diaphragm

Width of tissue Strip (mm)	Tensile Strength (Kg-Force) On Maxi Load & Break Strain						Elongation (%) On Maxi Load & Break Strain					
	Maxi Load			Break Strain			Maxi Load			Break Stain		
	Normal	ADM	Strength % of ADM	Normal	ADM	Strength % of ADM	Normal	ADM	Elongation strength of ADM	Normal	ADM	Elongation strength of ADM
5	0.72±0.04[a]	0.64±0.01[b]	88.89	0.80±0.01[c]	0.73±0.02[d]	91.25	25.37±0.53[e]	22.85±0.73[f]	90.07	26.81±0.34[g]	24.42±0.40[h]	91.09
10	1.18±0.04[a]	1.07±0.03[b]	90.68	1.44±0.05	1.36±0.01	94.44	36.13±0.48	35.41±1.10	98.01	38.53±0.67	37.25±1.03	96.68
20	4.026±0.13	3.72±0.07	91.85	4.98±0.09	4.75±0.06	95.38	39.71±0.64[e]	37.82±0.43[f]	95.24	43.18±0.46	42.41±0.60	98.21
40	6.27±0.08[a]	5.89±0.051[b]	93.94	8.33±0.08[c]	7.99±0.06[d]	95.91	42.35±0.87	41.69±0.79	98.44	44.61±0.64	43.73±0.46	98.02

Means superscripts with different letter differ significantly.

Table 2 (A): Anova table for tensile strength on maxi load and break strain

Source of Variation	Tensile Strength On Maxi Load (Kg-Force)					Tensile Strength On Break Strain (Kg-Force)				
	5 mm									
	DF	SS	MS	F-value	P-value	DF	SS	MS	F-value	P-value
Biomaterial	1	0.02	0.02	4.89*	$P<0.05$	1	0.02	0.02	18.75**	$P<0.0015$
Error	10	0.04	0.01			10	0.01	0.0008		
Corrected Total	11	0.06				11	0.02			
	10 mm									
Biomaterial	1	0.04	0.04	5.05*	$P<0.05$	1	0.02	0.02	1.91NS	0.20^{NS}
Error	10	0.07	0.01			10	0.08	0.01		
Corrected Total	11	0.11				11	0.10			
	20 mm									
Biomaterial	1	0.29	0.29	4.46NS	0.06^{NS}	1	0.15	0.15	4.65NS	0.056^{NS}
Error	10	0.65	0.06			10	0.33	0.03		
Corrected Total	11	0.93				11	0.48			
	40 mm									
Biomaterial	1	0.43	0.43	16.10*	P <.0025	1	1.53	1.53	54.63**	$P<.0001$
Error	10	0.26	0.03			10	0.28	0.028		
Corrected Total	11	0.69				11	1.81			

** Highly significant ($P<0.01$), *Significant ($p<0.05$), ^{NS}-Non-significant ($p>0.05$)

Table 2 (B): Anova table for elongation on maxi load and break strain

Source of variation	Elongation On Maxi Load (%)					Elongation On Break Strain (%)				
	5 mm									
	DF	SS	MS	F-value	P-value	DF	SS	MS	F-value	P-value
Biomaterial	1	19.10	19.10	7.87*	$P<0.019$	1	17.11	17.11	26.84**	$P<0.0004$
Error	10	24.28	2.43			10	6.38	0.64		
Corrected Total	11	43.38				11	23.49			
	10 mm									
Biomaterial	1	1.53	1.53	0.36NS	0.56	1	4.86	4.86	1.07NS	0.33
Error	10	42.93	4.29			10	45.39	4.54		
Corrected Total	11	44.46				11	50.25			
	20 mm									
Biomaterial	1	10.81	10.81	6.03*	$P<0.034$	1	1.80	1.80	1.04NS	0.33
Error	10	17.93	1.79			10	17.25	1.72		
Corrected Total	11	28.75				11	19.05			
	40 mm									
Biomaterial	1	1.31	1.31	0.32NS	0.59	1	2.27	2.27	1.23NS	0.29
Error	10	41.23	4.12			10	18.49	1.85		
Corrected Total	11	42.54				11	20.76			

** Highly significant ($P<0.01$), *Significant ($p<0.05$), ^{NS}-Non-significant ($p>0.05$)

Conclusion
In-vitro evaluation of biomechanical property of Acellular Diaphragm Matrix (ADM) of porcine origin was compared with fresh unprocessed diaphragm resulted satisfactorily excellent tensile strength as well as its tissue elongation property.

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