International Journal of Genetic Engineering and Biotechnology.
ISSN 0974-3073 Volume 2, Number 1 (2011), pp. 77-89

http://www.irphouse.com

Biochemical Changes in Rabbit Organs after Subcutaneous Implantation with Bovine Pericardium and Diaphragm

Himani Singh[a], Naveen Kumar[a], A.K. Sharma[a], Meena Katareia[b], and Ashok Munjal[c]

[a]Division of Surgery, [b]Division of Biochemistry,
Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India
[c]Department of Bioscience and Biotechnology,
Banasthali Vidyapith, 304022, Rajasthan, India.
Corresponding Author E-mail: h.singh2010@rediffmail.com,

Abstract

Native as well as acellular pericardial and diaphragmatic tissue of bovine was cross-linked with gluteraldehyde (GA) and hexamethylene diisocyanate (HMDC). These tissues were subcutaneously implanted in 39 rabbits. Biochemical parameter viz. free amino group content, protein content, acid phosphatase and alkaline phosphatase were evaluated in kidney, liver, lung and heart of all the rabbits at 90 post operative day. Normal rabbits were used as control.

Free amino group content, protein content, acid phosphatase and alkaline phosphatase activity significantly decreased in cross-linked groups as compared to uncross-linked groups in all the animals (native and acellular).In all implanted groups, HMDC cross-linked groups showed significantly lower values as compared to GA cross-linked groups. HMDC cross-linked biomaterials were found superior for the reconstructive surgery due to low free amino group, protein content, acid phosphatase and alkaline phosphatase activity as compared to GA cross-linked and uncross-linked biomaterials.

Keywords: Acellular, Cross-linking reagents, Proteins, Phosphtases, Kidney, Liver, Lung and Heart.

Abbreviations

ND-Native Diaphragm, AD-Acellular Diaphragm, NP-Native pericardium, AP-Acellular pericardium, GA-Glutaraldehyde, HMDC- Hexamethylene Diisocyanate, ACP- Acid Phosphtase, ALP- Alkaline Phosphtase.

Introduction

The biomaterials are used in close or direct contact with the body to augment or replace faulty materials. Biomaterial has been proven not to be completely inert after implantation and does generate an inflammatory response as a foreign body reaction that differs between individuals and depends on the amount of material and the structure of the mesh [1, 2, 3]. Enzyme digestion of hydrolysable bonds of implanted polymers is usually not observed [4, 5]. Williams (1977) demonstrated *in-vitro* that enzymes could increase the rate of degradation of several nominally stable polymers as, e.g., PETP, PMMA, Nylon 66 or a poly (etherurethane) [4, 6].

In general, two classes of enzymes are of interest to be studied in the immediate surroundings of the implantation site. First the hydrolases, hydrolytic enzymes like phoshatases, esterases, and amino peptidases. These enzymes are predominantly lysosomal and are mostly contained within macrophages and giant cells. The second class of enzymes is represented by the oxidoreductases, providing a way for further hydrolytic breakdown. It is interesting to know to what extent the cells in the immediate environment of an implanted biomaterial influence the *in-vivo* degradation, e.g., by the production of specific enzymes [7]. The concentrations of some intracellular enzymes as well as that of protein increases in the lymph draining a rabbit hind limb after the limb has been subjected to thermal or chemical injury, but the nature of the enzyme pattern depends upon the degree of cellular injury [8, 9]. The present study was carried out to study biochemical changes in rabbit organs after subcutaneous implantation with bovine diaphragm and pericardium as native and also in acellular form.

Materials and Methods

Fresh bovine pericardium and diaphragm were procured from local abattoir. Tissues were divided into two equal halves and one portion was used as native and another portion was made acellular as per the technique of Kumar (2009) [10]. Both native and acellular tissues were cross-liked using 5% gluteraldehyde (GA) and hexamethylene diisocyanate (HMDC). The tissues were treated with cross-links for 72 h.

Subcutaneous implantation of biomaterials in rabbits

Adult New Zealand white rabbits (39) of either sex were utilized for evaluation of biomaterials. The animals were kept off fed and water for 6 h and 12h respective before the implantation. The back of the animals was properly clipped, shaved and scrubbed with 5% cetrimide and chlorohexidine and painted with povidone iodine solution. The biomaterials were cut in 10x20 mm size and implanted in two pouches created on either side of the back. The animals (39) were randomly divided in to different groups of 3 animals each as shown in the table 1.

Table1: Subcutaneous implantation of biomaterials before and after cross-linking in different groups.

Groups	Sub Groups	No. of animals	Treatment given
Native Diaphragm (A)	A_1	3	Native Diaphragm (ND)
	A_2	3	Native Diaphragm cross-linked with GA
	A_3	3	Native Diaphragm cross-linked with HMDC
Acellular Diaphragm (B)	B_1	3	Acellular Diaphragm (AD)
	B_2	3	Acellular Diaphragm cross-linked with GA
	B_3	3	Acellular Diaphragm cross-linked with HMDC
Native Pericardium (C)	C_1	3	Native Pericardium (NP)
	C_2	3	Native Pericardium cross-linked with GA
	C_3	3	Native Pericardium cross-linked with HMDC
Acellular Pericardium (D)	D_1	3	Acellular Pericardium (AP)
	D_2	3	Acellular Pericardium cross-linked with GA
	D_3	3	Acellular Pericardium cross-linked with HMDC

ND-Native diaphragm, AD-Acellular diaphragm, NP-Native pericardium AP-Acellular pericardium, GA-Glutaraldehyde, HMDC- Hexamethylene diisocyanate.

Collection of organs

Immediately after euthanesia at day 90 postoperatively the organs (kidney, liver, lung and heart) were removed and chilled in crushed ice. The organs were thoroughly washed with cold deionized distilled water. The organs were then blotted on filter paper, cut into small pieces, weighed, and homogenized using a glass mortar and pestle in extraction buffer (50mM Tris, pH 7.4 containing 0.25% tritonX100 and 0.5% sodium dodecyle sulphate). The material was centrifuged at 3000 rpm for 10 min. After centrifugation the supernatants were separated with the help of pasture pipette and extracts were stored at - 20°C until used.

Biochemical studies

Following biochemical parameters were studied

1. Free amino group content

Ninhydrin assay was used to determine the free amino group content of each test sample, as per the procedure of Sung *et al*. (2000) [11].

2. Protein content
The protein contents in test sample homogenates were estimated by the method of Lowry *et al*. (1951) [12] using bovine serum albumin (BSA) as a standard.

3. Acid Phosphtase (ACP)
The determination of acid phosphatase activity in each test sample homogenates was based on the standered method of Kind and King's *et al.* (1934) [13].

Alkaline Phosphtase (ALP)
The determination of alkaline phosphatase activity in each test sample homogenates was based on the slandered method of Kind and King's *et al.* (1934) [13].

Statistical analysis
Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS Incorp, United States) was used for statistical analysis of the data. All experiments were conducted in triplicate to check the reproducibility of the results obtained. The results are presented as means ± SE (standard error) and means were compared using ANOVA followed by Bonferroni post-hoc analysis to observe the significant difference at $P<0.05$ level in different groups.

Results

Free amino group content
The free amino group concentration (mean ± SE) in different organs (kidney, liver, lung and heart) at day 90 postoperatively is presented in table 2.

Table2: Free amino group concentration (µg/ml) in different organs of rabbit after subcutaneous implantation of bovine diaphragm and pericardium at day 90.

Groups	Subgroups	Free amino acid concentration (µg/ml)			
		kidney	liver	lung	heart
Native Diaphragm (A)	ND (A_1)	36.33 ± 1.45^{A}	46.66 ± 1.33^{A}	40.33 ± 0.33^{A}	30.00 ± 0.57^{A}
	ND-GA (A_2)	34.33 ± 0.33^{A}	41.00 ± 2.08^{A}	37.00 ± 0.57^{B}	29.66 ± 1.20^{A}
	ND-HMDC(A_3)	30.33 ± 2.18^{B}	24.00 ± 2.18^{B}	22.33 ± 0.88^{C}	27.00 ± 1.52^{A}
Acellular Diaphragm (B)	AD (B_1)	25.33 ± 1.76^{C}	21.00 ± 1.57^{B}	18.00 ± 1.15^{D}	17.00 ± 0.57^{B}
	AD-GA (B_2)	23.33 ± 1.76^{D}	18.00 ± 1.15^{C}	16.66 ± 0.88^{D}	16.33 ± 0.88^{B}
	AD-HMDC(B_3)	21.00 ± 1.53^{D}	16.00 ± 0.58^{C}	13.00 ± 1.00^{E}	12.66 ± 0.33^{C}
Native Pericardium (C)	NP (C_1)	26.00 ± 1.15^{B}	34.66 ± 1.33^{B}	32.00 ± 0.58^{B}	29.00 ± 0.57^{B}
	NP-GA (C_2)	18.00 ± 0.58^{C}	30.66 ± 0.88^{B}	30.00 ± 0.58^{B}	23.66 ± 0.33^{C}
	NP-HMDC (C_3)	21.00 ± 0.58^{A}	28.00 ± 2.00^{C}	26.67 ± 0.66^{C}	20.33 ± 0.33^{C}

Acellular Pericardium (D)	AP (D_1)	22.33 ± 1.77^{A}	32.00 ± 1.15^{B}	28.00 ± 0.58^{C}	24.00 ± 0.58^{C}
	AP-GA (D_2)	12.33 ± 0.33^{D}	30.67 ± 0.66^{B}	25.33 ± 0.33^{C}	22.00 ± 0.58^{C}
	AP-HMDC(D_3)	15.66 ± 1.20^{D}	29.33 ± 0.66^{C}	21.33 ± 0.33^{D}	19.00 ± 0.58^{D}
	CONTROL	38.66 ± 1.48^{A}	48.33 ± 1.88^{A}	41.00 ± 0.58^{A}	34.33 ± 0.58^{A}

ABCDE-means bearing different superscripts in a column indicate the significant difference ($P<0.05$) among groups.ND-Native diaphragm, AD-Acellular diaphragm, NP-Native pericardium, AP-Acellular pericardium, GA-Glutaraldehyde, HMDC-Hexamethylene diisocyanate.

Among all diaphragm and pericardium implanted groups, uncross-linked groups (A_1, B_1, C_1 and D_1) showed significantly ($P<0.05$) increased values of free amino group content as compared to cross-linked groups (A_2, A_3, B_2, B_3, C_2, C_3, D_2 and D_3) in different organs (kidney, liver, lung and heart).

Kidney

Diaphragm groups: HMDC cross-linked groups (A_3, B_3) showed significant ($P<0.05$) decrease in free amino group content as compared to GA cross-linked groups (A_2, B_2). The highest free amino group content (36.33±1.45 mg/ml) was observed in native diaphragm group (A_1) and lowest free amino group content (21.00±1.53 mg/ml) was seen in acellular diaphragm group (B_3). The significant ($P<0.05$) decrease in free amino group content in A_3, B_1, B_2 and B_3 groups was observed as compared to control.

Pericardium groups: GA cross-linked groups (C_2, D_2) showed significant ($P<0.05$) decrease in free amino group content as compared to HMDC cross-linked groups (C_3, D_3). The highest free amino group content (26.00±1.15 mg/ml) was observed in native pericardium group (C_1) and lowest value (12.33±0.33 mg/ml) was observed in GA cross-linked acellular pericardium group (D_2). The significant ($P<0.05$) decrease in free amino acid content in C_1, C_2, D_2 and D_3 groups was observed as compared to control.

Liver

Diaphragm groups: HMDC cross-linked groups (A_3, B_3) showed significant ($P<0.05$) decrease in free amino group contents as compared to GA cross-linked groups (A_2, B_2). The highest free amino acid content (46.66±1.33 mg/ml) was observed in uncross-linked native diaphragm group (A_1) and lowest value (16.00±0.58 mg/ml) was observed in HMDC cross-linked acellular diaphragm group (B_3). The significant ($P<0.05$) decrease in free amino group content was observed in A_3, B_1, B_2 and B_3 groups as compared to control.

Pericardium groups: The pericardium implanted groups showed similar results as in diaphragm implanted groups. The highest free amino group content (34.66±1.33 mg/ml) was observed in native pericardium group (C_1) and lowest value (28.00±2.00 mg/ml) was observed in HMDC cross-linked native pericardium group (C_3). The significant ($P<0.05$) decrease in free amino group was observed in all implanted groups as compared to control.

Lung

Diaphragm groups: GA cross-linked group (A_2, B_2) showed significant ($P<0.05$) increased in free amino group content as compared to HMDC cross-linked groups (A_3, B_3). The highest value (40.33±0.33 mg/ml) was observed in uncross-linked native diaphragm group (A_1) and lowest value (13.00±1.00 mg/ml) in HMDC cross-linked acellular diaphragm group (B_3). The significant ($P<0.05$) decreased in free amino group content was observed in all implanted groups except uncross-linked native diaphragm group (A_1) as compared to control.

Pericardium groups: The pericardium implanted groups showed similar results as diaphragm implanted groups. The highest value (32.00±0.58 mg/ml) was observed in native pericardium group (C_1) and lowest (21.33±0.33 mg/ml) in HMDC cross-linked acellular pericardium group (D_3). The free amino group was significantly ($P<0.05$) decreased in all implanted groups as compared to control.

Heart

Diaphragm groups: GA cross-linked groups (A_2, B_2) showed significant ($P<0.05$) increased of free amino group content as compared to HMDC cross-linked groups (A_3, B_3). The highest free amino group (30.00±0.57 mg/ml) content was observed in uncross-linked native diaphragm group (A_1) and lowest (12.66±0.33 mg/ml) in HMDC cross-linked acellular diaphragm group (B_3). Uncross-linked and cross-linked acellular diaphragm groups (B_1, B_2 and B_3) showed significantly ($P<0.05$) decrease in amino group as compared to control.

Pericardium group: In pericardium implanted groups, the results were similar as in diaphragm implanted groups. The highest free amino group content (29.00±0.57 mg/ml) was seen in uncross-linked native pericardium group (C_1) and lowest (19.00±0.58 mg/ml) in HMDC cross-linked acellular diaphragm group (D_3). The free amino group content was found significantly ($P<0.05$) lower in all implanted groups in comparison to control.

Protein concentration

The protein concentration (mean ± SE) in different organs (kidney, liver, lung and heart) is presented in table 3.

Table3: Free protein concentration (mg/ml) in different organs of rabbit after subcutaneous implantation of bovine diaphragm and pericardium at day 90.

Groups	Subgroups	Free protein concentration (mg/ml)			
		Kidney	Liver	Lung	heart
Native Diaphragm (A)	ND (A_1)	1.54 ± 0.03^{C}	0.86 ± 0.02^{B}	0.87 ± 0.02^{B}	0.93 ± 0.01^{B}
	ND-GA (A_2)	1.44 ± 0.03^{B}	2.46 ± 0.04^{D}	2.71 ± 0.01^{D}	1.39 ± 0.01^{C}
	ND-HMDC(A_3)	1.17 ± 0.02^{D}	1.21 ± 0.01^{C}	1.17 ± 0.01^{C}	2.45 ± 0.02^{D}
Acellular	AD (B_1)	1.55 ±	0.98 ± 0.01^{B}	0.88 ± 0.02^{B}	1.18 ± 0.02^{C}

Diaphragm (B)		0.03^{C}			
	AD-GA (B_2)	1.45 ± 0.01^{B}	2.43 ± 0.04^{D}	2.33 ± 0.02^{D}	2.18 ± 0.01^{D}
	AD-HMDC(B_3)	1.43 ± 0.03^{B}	2.17 ± 0.01^{D}	1.92 ± 0.02^{E}	2.20 ± 0.06^{D}
Native Pericardium (C)	NP (C_1)	0.64 ± 0.02^{C}	2.26 ± 0.02^{B}	2.26 ± 0.02^{B}	2.19 ± 0.01^{B}
	NP-GA (C_2)	0.53 ± 0.04^{C}	2.19 ± 0.01^{B}	2.06 ± 0.02^{B}	2.12 ± 0.00^{B}
	NP-HMDC (C_3)	1.62 ± 0.01^{A}	1.80 ± 0.01^{C}	1.85 ± 0.02^{C}	1.79 ± 0.01^{C}
Acellular Pericardium (D)	AP (D_1)	0.58 ± 0.01^{C}	2.21 ± 0.04^{B}	2.21 ± 0.06^{B}	2.18 ± 0.00^{B}
	AP-GA (D_2)	0.40 ± 0.01^{C}	2.15 ± 0.02^{B}	2.19 ± 0.02^{B}	2.12 ± 0.00^{B}
	AP-HMDC (D_3)	1.45 ± 0.01^{B}	2.11 ± 0.04^{B}	2.18 ± 0.01^{B}	2.10 ± 0.01^{B}
	CONTROL	1.65 ± 0.08^{A}	0.62 ± 0.04^{A}	0.57 ± 0.02^{A}	0.69 ± 0.02^{A}

ABCDE-means bearing different superscripts in a column indicate the significant difference ($P<0.05$) among groups. ND-Native diaphragm, AD-Acellular diaphragm, NP-Native pericardium, AP-Acellular pericardium, GA-Glutaraldehyde, HMDC-Hexamethylene diisocyanate.

Kidney

Diaphragm groups: The protein content was found significantly ($P<0.05$) higher in uncross-linked groups (A_1, B_1) as compared to cross-linked groups (A_2, A_3 and B_2, B_3). HMDC cross-linked groups (A_3, B_3) showed significant ($P<0.05$) decrease in protein as compared to GA cross-linked groups (A_2, B_2). The protein content was significantly ($P<0.05$) decreased in all diaphragm implanted groups as compared to control.

Pericardium groups: The protein content was found significantly ($P<0.05$) increased in HMDC cross-linked groups (C_3, D_3) as compared to uncross-linked and GA cross-linked groups (C_1,C_2 and D_1,D_2). However, the values in implanted groups were significant ($P<0.05$) lower as compared to control.

Liver

Diaphragm groups: The protein content was significantly ($P<0.05$) lower in uncross-linked groups (A_1, B_1) as compared to cross-linked groups (A_2, A_3 and B_2, B_3). HMDC cross-linked groups (A_3, B_3) showed significantly ($P<0.05$) decrease value as compared to GA cross-linked groups (A_2, B_2). The protein content remained significantly ($P<0.05$) increase in all implanted groups in comparison to control.

Pericardium group: Uncross-linked groups (C_1, D_1) showed significant ($P<0.05$) increased values as compared to cross-linked groups (C_2, C_3 and D_2, D_3). HMDC cross-linked groups (C_3, D_3) showed significant ($P<0.05$) decrease values ($P<0.05$) of protein as compared to GA cross-linked groups (C_2, D_2). The protein content remained significantly ($P<0.05$) higher in all implanted groups in comparison to control.

Lung

Diaphragm group: Protein content showed significant (P<0.05) decrease in uncross-linked groups (A_1, B_1) as compared to cross-linked groups (A_2, A_3 and B_2, B_3). GA cross-linked groups (A_2, B_2) showed significant (P<0.05) increase in protein content as compared to HMDC cross-linked groups (A_3, B_3). Protein content remained significantly (P<0.05) increased in diaphragm implanted groups as compared to control.

Pericardium group: Uncross-linked groups (C_1, D_1) showed significant (P<0.05) increase in protein content as compared to cross-linked groups (C_2, C_3 and D_2, D_3). HMDC cross-linked groups (C_3, D_3) showed significant (P<0.05) decrease in protein content as compared to GA cross-linked groups (C_2, D_2).However the protein content remained significantly (P<0.05) higher in all implanted groups in comparison to control.

Heart

Diaphragm group: Uncross-linked groups (A_1, B_1) showed significant (P<0.05) decrease in protein content as compared to cross-linked groups (A_2, A_3 and B_2, B_3). HMDC cross-linked groups (A_3, B_3) showed significant (P<0.05) increase in values of protein as compared to GA cross-linked groups (A_2, B_2). Protein content was significantly (P<0.05) increased in diaphragm implanted groups as compared to control group.

Pericardium group: Uncross-linked groups (C_1, D_1) showed significant (P<0.05) increase in protein content as compared to cross-linked groups (C_2, C_3 and D_2, D_3). GA cross-linked groups (C_2, D_2) showed significant (P<0.05) increase in protein content as compared to HMDC cross-linked groups (C_3, D_3). The protein content remained significantly (P<0.05) increased in all implanted groups in comparison to control.

Acid and alkaline phosphatase

The acid phosphatase and alkaline phosphatase activity (mean ± SE) in different organs (kidney, liver, lung and heart) after euthanesia on day 90 postoperatively is presented in table 4 and 5.

Table4: Acid phosphate (U/L) in different organs of rabbit after subcutaneous implantation of bovine diaphragm and pericardium at day 90.

Groups	Subgroups	Acid phosphatase activity (U/L)			
		Kidney	Liver	Lung	heart
Native Diaphragm (A)	ND (A_1)	65.45 ± 6.71^{D}	70.12± 8.21^{D}	68.23± 9.41^{D}	60.12± 9.76^{D}
	ND-GA (A_2)	61.36± 2.96^{C}	69.22± 5.33^{B}	64.21± 8.75^{B}	57.34± 5.51^{B}
	ND-HMDC(A_3)	59.25 ± 4.40^{B}	64.32± 7.61^{C}	63.43± 4.54^{B}	56.12± 8.91^{B}
Acellular	AD (B_1)	60.59 ±	68.15±	66.03±	58.32±

Diaphragm (B)		7.02^{C}	9.22^{B}	7.45^{C}	12.8^{C}
	AD-GA (B_2)	58.96 ± 10.2^{B}	67.32± 6.09^{B}	63.21± 6.81^{B}	56.32± 7.45^{B}
	AD-HMDC(B_3)	57.02 ± 4.42^{B}	65.87± 4.01^{B}	62.10± 4.89^{B}	55.11± 9.38^{B}
Native pericardium (C)	NP (C_1)	63.16 ± 5.40^{D}	68.98± 6.73^{D}	65.12± 9.50^{D}	59.12± 9.47^{D}
	NP-GA (C_2)	58.16 ± 3.34^{C}	66.23± 4.53^{C}	63.20± 7.58^{C}	55.56± 11.3^{C}
	NP-HMDC (C_3)	56.83 ± 12.6^{B}	64.12± 8.96^{B}	60.80± 6.43^{B}	53.23± 6.58^{B}
Acellular pericardium (D)	AP (D_1)	58.16 ± 3.12^{C}	65.23± 9.34^{C}	62.10± 10.4^{C}	57.43± 9.70^{C}
	AP-GA (D_2)	55.10 ± 8.62^{B}	63.21± 4.92^{B}	60.13± 6.59^{B}	54.21± 6.91^{B}
	AP-HMDC (D_3)	53.60 ± 12.6^{B}	62.89± 5.41^{B}	59.35± 8.73^{B}	53.10± 9.01^{B}
	CONTROL	48.80 ± 10.1^{A}	54.29± 6.90^{A}	51.65± 9.04^{A}	47.34± 10.5^{A}

ABCDE-means bearing different superscripts in a column indicate the significant difference ($P<0.05$) among groups. ND-Native diaphragm, AD-Acellular diaphragm, NP-Native pericardium, AP-Acellular pericardium, GA-Glutaraldehyde, HMDC-Hexamethylene diisocyanate.

Table5: Alkaline phosphate (U/L) in different organs of rabbit after subcutaneous implantation of bovine diaphragm and pericardium at day 90.

Groups	Subgroups	Alkaline phosphatase activity (U/L)			
		Kidney	Liver	Lung	heart
Native Diaphragm (A)	ND (A_1)	39.02± 5.05^{C}	61.23± 4.31^{D}	57.87± 6.90^{C}	30.12± 12.6^{D}
	ND-GA (A_2)	37.23 ± 5.15^{C}	59.21± 8.72^{D}	56.34± 8.20^{C}	28.10± 12.6^{C}
	ND-HMDC(A_3)	36.82± 1.00^{B}	58.90± 6.30^{C}	55.12± 10.6^{B}	27.18± 12.6^{B}
Acellular Diaphragm (B)	AD (B_1)	38.00 ± 2.17^{C}	57.38± 5.03^{C}	55.67± 9.75^{B}	29.19± 12.6^{C}
	AD-GA (B_2)	35.86 ±3.04^{B}	56.13± 6.82^{C}	54.34± 11.2^{B}	27.10± 12.6^{B}
	AD-HMDC(B_3)	34.92± 2.70^{B}	54.78± 5.33^{B}	53.65± 9.06^{B}	26.19± 12.6^{B}

Native pericardium (C)	NP (C_1)	36.43 ± 4.25^{C}	56.89± 7.42^{C}	54.76± 7.56^{C}	27.32± 12.6^{C}
	NP-GA (C_2)	35.53 ±7.64^{C}	54.29± 4.60^{C}	53.98± 6.04^{C}	26.13± 12.6^{C}
	NP-HMDC (C_3)	34.36 ± 5.99^{B}	53.34± 8.83^{B}	52.21± 6.64^{C}	24.16± 12.6^{B}
Acellular pericardium (D)	AP (D_1)	35.13 ± 8.60^{C}	55.32± 10.2^{C}	53.54± 7.23^{C}	26.52± 12.6^{C}
	AP-GA (D_2)	34.93 ± 8.05^{B}	52.48± 2.61^{B}	50.76± 8.21^{B}	25.17± 12.6^{C}
	AP-HMDC (D_3)	32.56 ± 1.42^{B}	51.98± 9.60^{B}	49.82± 9.42^{B}	23.67± 12.6^{B}
	CONTROL	28.40 ± 5.31^{A}	47.65± 10.6^{A}	44.45± 5.34^{A}	20.89± 12.6^{A}

ABCDE-means bearing different superscripts in a column indicate the significant difference ($P<0.05$) among groups. ND-Native diaphragm, AD-Acellular diaphragm, NP-Native pericardium, AP-Acellular pericardium, GA-Glutaraldehyde, HMDC-Hexamethylene diisocyanate.

In all diaphragm and pericardium implanted groups, uncross-linked groups (A_1, B_1, C_1, D_1) showed significant ($P<0.05$) increase as compared to cross-linked groups (A_2, A_3, B_2, B_3 and C_2, C_3, D_2, D_3) in different organs (kidney, liver, lung and heart).

HMDC cross-linked groups (A_3, B_3 and C_3, D_3) showed significant ($P<0.05$) decrease in acid phosphatase and alkaline phosphatase activity in all the four organs (kidney, liver, lung and heart) of diaphragm and pericardium implanted rabbit groups as compared to GA cross-linked groups (A_2, B_2 and C_2, D_2). The enzyme activity was significantly ($P<0.05$) increased in all implanted groups as compared to control. Highest activity was observed in native diaphragm implanted group and lowest value of acid phosphatase and alkaline phosphatase activities were recorded in HMDC cross-linked acellular pericardium implanted group.

Discussion

Woolf, 1976 and Dale, 1977 [14, 15] reported a postoperative fall of plasma amino acids. However, the mechanism by which surgical operation results in a lowered concentration of plasma amino acids has not been known. It has been suggested that postoperative malnutrition is the main causative factor [16]. Free amino group (mg/ml) significantly decreased in all implanted groups as compared to control in different organs (kidney, liver, lung and heart). In diaphragm and pericardium implanted groups uncross-linked groups (native and acellular) showed significantly increase as compared to cross-linked groups. In all implanted groups, free amino group was significantly lower in HMDC cross-linked groups as compared to GA cross-linked groups in liver, lung, heart. Where as only kidney extract of pericardium implanted groups exhibited significantly increase value of amino acid concentration in

HMDC cross-linked groups as compared to GA cross-linked groups. The reduction of free amino groups (determined by ninhydrin assay in the present study) in biological tissue diminishes it anitigenicity [17]. Wu and Bauer (1960) observed a relatively large increase in the plasma free amino acid levels to accompany tumor growth [18]. Norten *et al.,* (1985) have shown that cancer patients with no or minimum body weight loss maintained their plasma free amino acid levels within normal range [19].

Protein concentration in kidney, liver and lung was found significantly increased in GA cross-linked diaphragm implanted groups as compared to HMDC cross-linked groups. Whereas heart showed significantly increase in HMDC cross-linked group as compared to GA cross-linked group. All pericardium implanted groups showed significantly increased protein contents in GA cross-linked groups as compared to HMDC cross-linked groups in liver, lung and heart but low protein contents in kidney of GA cross-linked groups was observed. Hypoalbuminemia could have a profound effect on the response of patients to surgery. This effect has been investigated in experimental animals and in man. Impaired wound healing [20] increased susceptibility to hemorrhagic shock [21] and increased incidence of postoperative infection [22] have long been known to be associated with hypoalbuminemia. More recently, impaired immune function has been reported in surgical patients with low plasma albumin levels [23].

The enzymes have been differentiated in various tissues and in serum on the basis of their inhibition by different physical and chemical treatments. Decreased serum alkaline phosphatase activity has been observed in infants who are undergoing cardiac surgery with profound hypothermia, circulatory arrest, and limited cardiopulmonary bypass [24]. Total alkaline phosphatase activity in sera reflects a number of alkaline phosphohydrolases of several tissue origins. Tissue-specific alkaline phosphatases have been identified in bone, liver, intestine, and placenta [25, 26]. The increase levels of alkaline phosphatase in traumatic area as a result of tissue injuries have been reported to help in the proliferation of fibroblasts [27]. Alkaline phosphatase appeared to be associated with the metabolic process concerning collagen formation [28].Acid phosphatase and alkaline phosphatase activity was significantly decreased in HMDC cross-linked groups as compared to GA cross-linked groups in different organs (kidney, liver, lung and heart) of diaphragm and pericardium implanted groups. In the healing process of wound, various tissue enzymes play crucial role. The alkaline phosphatase (a zinc containing enzymes) is located in cell membrane of various cells (neutrophils, macrophages giant cells, fibroblasts) of the body. Alkaline phosphatase activity was markedly affected by the tumor and associated with various metabolic processes of bone, and its blood level provides valuable means in the diagnosis of bone disease [29]. Alkaline phosphatase activity in kidney, bone, intestine, and liver provides a sensitive biochemical index for monitoring any change in the cellular activities of these organs.

In conclusion our results indicated that free amino acid concentration, protein concentration, acid phosphatase and alkaline phosphatase activity were observed significantly decreased in HMDC cross-linked groups as compared to GA cross-linked and native groups in different organs (kidney, liver, lung and heart) of diaphragm and pericardium implanted groups. The conclusion may be made that

HMDC cross-linked acellular pericardium and diaphragm are best biomaterials for the reconstructive surgery in animals.

Acknowledgement

The authors acknowledge the financial assistance received from the Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi, India to carry out this research work.

References

[1] Klosterhalfen B., Klinge U. and Hermanns B., 2000, "Pathology of traditional surgical nets for hernia repair after long-term implantation in humans," Chirurg.,71, pp. 43–51.
[2] Coda A., Bendavid R. and Botto-Micca F., 2003, "Structural alterations of prosthetic meshes in humans," Hernia., 7, pp. 29–34.
[3] Schachtrupp A., U. Klinge and K. Junge et al., 2003," Individual inflammatory response of human blood monocytes to mesh biomaterials," Br J Surg., 90, pp.114–120.
[4] G. E. Visser, R. L. Robison, H. V. Maulding, J. W. Fong, J. E. Pearon, and G. J. Argentieri., 1986, "Note: Biodegradation of and tissue reaction to poly-(DL-lactide) microcapsules," J. Biomed. Muter. Res., 20, pp. 667-676.
[5] T. N. Salthouse and B. F. Matlaga., 1976, "Polyglactin 910 suture absorption and the role of cellular enzymes," Surg. Gynaecol. Obstet., 142, pp. 544-550.
[6] D. F. Williams and E. Mort, 1977, "Enzyme- accelerated hydrolysis of polyglycolic acid," J. Bioeng.,1, pp.231-238.
[7] D. F. Williams, et al.,1979, "Some observations on the role of cellular enzymes in the in vivo degradation of some polymers," in Corrosion of implant Materials, B. Syvett and A. Achyara (eds.), ASTM, Philadelphia., pp. 61.
[8] Lewis, G. P., 1969, "Changes in the composition of rabbit hind limb lymph after thermal injury," J. Physiol., Lond., 205, pp.619-634.
[9] Boyles, Susan, Lewis, G. P.and Westcott, Barbara.,1970,"Changes in the composition of hind limb lymph after chemical injury," Br. J. Pharmac., 39, pp.798-808.
[10] Amit Kumar., 2009, "Biocompatibility of cross-linked pericardium and diaphragm of caprine origin," MVSc thesis submitted to deemed university, IVRI, Izatnagar, Uttarpradesh.
[11] Sung, H.W.,Chang, Y., Liang, I.L., Chang, W.H., Chen, U.C., 2000, "J Biomed Mater Res.," 52, pp.77-87.
[12] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., 1951, "J Biol Chem.,"193, pp. 265-275.
[13] King E.J. and Armstrong A.R., 1934, "Plasma alkaline phosphtase in disease," Can. Med. Ass. J., 31, pp. 376.

[14] Woolf L.I., Groves A.C., Moore J.P., Duff J.M., Finley R.J., Loomer R.L.,1976, "Arterial plasma amino acids in patients with serious postoperative infection and in patients with major fractures," Surgery., 79, pp. 283-292.
[15] Dale G., Young F., Latner A.L., Goode A., Tweedle D., Johnston I.D.A.,1977, " The effect of surgical operation on venous plasma free amino acids," Surgery., 81, pp. 295-301.
[16] Schonheyder F., Bon J., Skjoldborg H.,1974,"Variations in plasma amino acid concentrations after abdominal surgical procedures," Acta Chir Scand., 140, pp. 271-275.
[17] Imamura, E., Sawatani, O., Koyanagi, H., Noishiki, Y. and Miyata, T., 1989, "Epoxy compounds as a new crosslinking agent for porcine aortic leaflets: subcutaneous implants studies in rats," J Cardiac Sur., 4, pp.50-57.
[18] Wu C., Bauer J.M., 1960, "A study of free amino acids and of glutamine synthesis in tumor bearing rats," Cancer Res., 20, pp. 848-856.
[19] Norton J.A., Gorschboth C.M., Wesley R.A., Burt M.E., Brennan M.F.,1985, "Fasting plasma amino acid levels in cancer patients," Cancer., 56, pp.1181-1186.
[20] Thompson W.D., Ravdin I.S., Frank I.L., 1938, "Effect of hypoproteinemia on wound disruption," Arch Surg., 36, pp. 500-518.
[21] Ravdin I.S., Mcnamee H.G., Kamholz J.H., Rhoads J.E., 1944, "Effect of hypoproteinemia on susceptibility to shock resulting from hemorrhage," Arch Surg., 48, pp. 491-497.
[22] Rhoads J.E., Alexander C.F.,1955, " Nutritional problems of surgical patients," Ann NYAcad Sci., 63, pp. 268-275.
[23] Law D.K., Dudrick S.J., Abdou N.I., 1974, "The effects of protein calorie malnutrition on immune competence of the surgical patient," Surg Gynecol Obstet., 139, pp. 257-266.
[24] Neutze J.M., Drakeley M.J., Barratt-Boyes B.G.,1974, "Serum enzymes after cardiac surgery under profound hypothermia with circulatory arrest and limited cardiopulmonary bypass," Am Heart J., 88, pp. 553-556.
[25] Fishman, W. H., and N. K. Ghosh. 1967, "Isozymes of human alkaline phosphatas," Advan. Clin. Chem., 10, pp. 255.
[26] Posen, S. 1967, "Alkaline phosphatase," Ann. Intern. Med., 67, pp. 183.
[27] Soni, N. K., Patel, M. R. and Shrivastava, R.K.1976, "Alkaline phosphatase, total protein, protein bound hexoses and hexosamine changes in serum and wound fluids in surgically induced subcutaneous wounds in buffalo calves," Acta Vet Acad Sci Hung., 26 (3), pp. 263-270.
[28] French, J. E. and Beneditt, E. P., 1954, "Observations on the localization of alkaline phosphates in healing wound," Arch. Path., 57, pp.352-356.
[29] King, E. J., and Moss, D. W., 1962, "Enzymes in Metabolic Bone Disease," In: H. A. Sissons (ed.), Bone Metabolism in Relation to Clinical Medicine., pp. 42--54. London: Pitman Medical Publishing Co.