CHAPTER V
SUMMARY AND CONCLUSIONS

Large abdominal hernias present an ongoing challenge to reconstructive surgery. Although several repair techniques have been described, synthetic materials are currently being investigated but failed to elicit biological cues similar to biological matrices. However, native biological materials tend to immunogenic and hence are decellularized to minimize their immunogenicity. In light of this background, present clinical study was conducted with two objectives viz. 1. To standardize preparation of decellularized aortic and diaphragmatic matrices of buffalo origin, and 2. To evaluate biocompatibility of prepared matrices for the repair of clinical abdominal hernias in cattle.

Fresh aorta of buffalo origin was decellularized using established protocol (1 % SDS for 24 h, 0.25 % trypsin for 2 h and again 1 % SDS for 24 h), whereas fresh tendinous diaphragm of buffalo origin with different concentrations of sodium dodecyl sulphate (SDS) (0.5 %, 1.0 %, 2 %, 3 % and 4 %) over a range of periods (12 h, 24 h, 48 h and 72 h) at 37 °C under physical agitation. Decellularization completeness was confirmed histologically. Prepared matrices were further characterized by Masson’s trichrome and Weigert staining, scanning electron microscopy (SEM) and DNA quantification. Twelve Gir cattle (eight females and four males) weighing from 35 to 550 kg (average weight 199.16 kg [DAM (I) group] and, 322.5 kg [DDM (II) group] aged between 1 and 18 months with abdominal hernias were assigned into two equal groups: implanted with decellularized aortic matrix (DAM) and decellularized diaphragmatic matrix (DDM). Clinical, hematological, biochemical and circulating antioxidants evaluation was carried on days 0, 7 and 15 to assess healing progress.

Histologically, absence of cells and orderly arranged collagen fibres were observed in aorta treated with 1 % SDS for 24 h followed by 0.25 % trypsin for 2 h and again with 1 % SDS for 24 h, and diaphragm treated with 2 % SDS for 48 h. Further, Masson’s trichrome and Weigert’s resorcin fuschin staining of tissue section confirmed intact collagen and elastic fibers within decellularized matrices. SEM examination confirmed preservation of collagen fibrils arrangement within prepared
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Matrices. DNA concentration was 488.11 ± 49.12 ng/mg (361.41-601.88 ng/mg), 443.95 ± 162.60 ng/mg (12.74-808.92 ng/mg), 6.47 ± 1.26 ng/mg (325.38-637.90 ng/mg) and 33.13 ± 5.39 ng/mg (106.28-927.94 ng/mg) in native aorta, native diaphragm, DAM and DDM respectively. DNA content highly significantly ($P < 0.001$) decreased in DAM as compared to native aorta whereas DNA content of DDM was significantly ($P < 0.05$) decreased as compared to native diaphragm. Treatment with 1 % SDS for 24 h/0.25 % trypsin for 2 h/1 % SDS for further 24 h resulted in 98.68 % reduction in DNA content of the aorta. Treatment with 2 % SDS for 48 h resulted in 92.54 % reduction in DNA content of the diaphragm. On agarose gel electrophoresis, dark intact DNA bands were observed in lane 1 (native aorta) and lane 3 (native diaphragm), and were absent in lane 2 (DAM) and lane 4 (DDM) indicating, no DNA content was present in prepared decellularized matrices. In FTIR analysis, the amide A band was found at 3302.24 cm\(^{-1}\) for native aorta, 3386.15 cm\(^{-1}\) for native diaphragm, 3340.82 cm\(^{-1}\) for DAM, 3343.71 cm\(^{-1}\) for DDM and 3285.88 cm\(^{-1}\) for hydroxyproline standard. Amide B band is related to an asymmetrical stretch of CH\(_2\) and was observed at 3067.88 cm\(^{-1}\) for native aorta, 3062.10 cm\(^{-1}\) for native diaphragm, 3057.27 cm\(^{-1}\) for DAM, 3081.39 cm\(^{-1}\) for DDM and 3091.03 cm\(^{-1}\) for hydroxyproline standard. The amide I band was recorded at 1658.84 cm\(^{-1}\) for native aorta, 1664.72 cm\(^{-1}\) for native diaphragm, 1665.59 cm\(^{-1}\) for DAM, 1649.19 cm\(^{-1}\) for DDM and 1640.51 cm\(^{-1}\) for hydroxyproline standard. The amide II of native aorta, native diaphragm, DAM, DDM and hydroxyproline standard appeared at 1526.71 cm\(^{-1}\), 1535.39 cm\(^{-1}\), 1529.60 cm\(^{-1}\), and 1597.11 cm\(^{-1}\), 1584.77 cm\(^{-1}\) respectively. The amide III band was found at 1282.55 cm\(^{-1}\) for native aorta, 1284.73 cm\(^{-1}\) for native diaphragm, 1230.69 cm\(^{-1}\) for DAM, 1220.02 cm\(^{-1}\) for DDM and 1283.67 cm\(^{-1}\) for hydroxyproline standard confirming presence of hydrogen bonds.

Rectal temperature, heart and respiration rates were within normal range at the time of presentation and on postoperative days 7 and 15 in animals of both DAM (I) and DDM (II) groups. Hernial ring size varied from 12.04 to 380.13 cm\(^2\) and intestine was the most commonly found hernial content. Postoperatively, in animals of DAM (I) and DDM (II) groups, inflammatory edema with mild somatic pain on palpation, warmth and exudation was observed during the first 3 post-implantation days and reduced gradually till complete resolution in all cases. In both groups, one animal each developed a large abscess at implantation site, which was subsided on post-operative day 15. Similarly, in both group, one animal each showed huge, painful
swelling at urethral region up to post-operative day 7, which was reduced in size on post-operative day 15. No postoperative complications were observed after retroperitoneal placement of DAM and DDM. No swelling at the wound side was observed at the time of removal of skin suture (post-operative day 15) in animals of both groups. All wounds healed by first intention healing. A thick and slightly hard mass was palpated at the site of the hernioplasty up to two weeks post-implantation, further subsided and region looked normal on inspection. All animals had an eventful recovery without clinical signs of wound dehiscence or reoccurrence of hernia, except for inflammatory edema and abscess in one case each in both the groups.

We observed significant ($P < 0.05$) decrease in erythrocyte sedimentation rate (ESR) and no significant ($P > 0.05$) changes in parameters viz. hemoglobin (Hb), packed cell volume (PCV), lymphocyte percent, monocyte percent, eosinophil percent and basophil percent in both the groups. A significant ($P < 0.05$) increase found in the total leukocyte and total erythrocyte counts in group DDM (II) and DAM (I), respectively, while a significant ($P < 0.05$) decrease was observed in neutrophil percent on postoperative day 15 in group DDM (II).

Biochemical parameters including total protein (TP), albumin, globulin, albumin-globulin (A:G) ratio, glucose, creatinine, urea, urea nitrogen, cholesterol, triglyceride, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), and gamma-glutamyl transferase (GGT) activities values were non-significantly ($P > 0.05$) changed between days 0, 7 and 15 in both groups. Creatinine level was significantly ($P < 0.05$) lower on day 7 in group DDM (II) as compared to group DAM (I). However, this value was well within normal reference range.

On erythrocytic antioxidant evaluation, both catalase and reduced glutathione levels were non-significantly ($P > 0.05$) changed between days 0, 7 and 15 in both groups.

**Conclusions**

1. A three step standardized protocol [(i) 1 % SDS for 24 h (ii) 0.25 % trypsin for 2 h (iii) 1 % SDS for 24 h] can be used to decellularize aorta of bubaline origin.

2. A single step standardized protocol (using 2 % SDS for 48 h) can be used to decellularize diaphragm of bubaline origin.
3. Histologically, native diaphragm treated with 2 % SDS for 48 h revealed effective removal of cellular components without altering collagen lattice scaffold.

4. SEM imaging revealed intact collagen within prepared extra cellular matrix scaffolds.

5. Peaks of FTIR spectra of decellularized aortic and diaphragmatic matrices are of collagen lattice within the prepared scaffolds.

6. Treatment with 1 % SDS for 24 h, 0.25 % trypsin for 2 h and 1 % SDS for further 24 h resulted in 98.68 % reduction in DNA content of the aorta. Treatment with 2 % SDS for 48 h resulted in 92.54 % reduction in DNA content of the diaphragm.

7. Prepared DAM and DDM of bubaline origin are biocompatible as evident by clinical, hematological, biochemical and antioxidant evaluation. Hence, DAM and DDM of bubaline origin can be safely used for hernioplasty in cattle.