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Decellularisation of tunica vaginalis for the production of tissue engineered scaffolds: A promising approach in tissue engineering

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Abstract

Collagen membranes are one of the most commonly used tissue engineered scaffold. Collagen scaffolds could be effectively made by decellularising a variety of biological tissues like small intestinal submucosa, dermis, omentum, tunica vaginalis, etc. These scaffolds are effectively used for the reconstruction of hernial defects, oesophageal defects, urinary bladder wall substitution, as artificial periosteum, etc. Decellularisation can be done using different methods like physical, chemical, and enzymatic. The present study discusses the successful decellularisation of bovine tunica vaginalis by combining enzymatic and chemical methods using the Trypsin-EDTA protocol with Triton X-100 as a detergent and using it as a tissue engineered scaffold.

Keywords: *Collagen scaffolds, decellularisation, bovine tunica vaginalis, Trypsin-EDTA protocol*

Collagen membrane is the most widely used tissue engineered scaffold used in regenerative medicine (Padmanabhan *et al.,* 2015). The main advantages of using collagen scaffolds includes its haemostatic property which helps in early wound stabilisation as well as its ability to attract fibroblasts. Tunica vaginalis is a double-layered structure that covers the testis,

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with the exception of the posterior and superior borders, which signifies the connection of the epididymis and spermatic cord (Titi-Lartey and Khan, 2020). Collagen membrane can be produced from this tough layer by a process of defattening followed by decellularisation. Scaffolds processed in this way from the tunica vaginalis were used earlier to repair complete thickness abdominal wall lesions in a rat model (Hafeez *et al*., 2005), urinary bladder wall defects in dogs (Wongsetthachai *et al*., 2011), perineal hernia defects in dogs (Guerios *et al*., 2020; Pratummintra *et al*., 2012) and partial oesophageal defects in dogs (Hashem *et al*., 2023). A method of decellularisation for the preparation of collagen scaffold from bovine tunica vaginalis for its successful use as tissue engineered scaffold has been discussed.

Fresh tunica vaginalis was isolated from the testes of adult bovines that were processed at the Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy (Fig.1-2). The collagen scaffold was produced from the parietal layer of the tunica vaginalis using a two-step approach involving defattening and subsequent decellularisation. In the defattening phase, the separated tunica vaginalis was submerged in a solution comprising chloroform and methanol in a 2:1 ratio, and it was allowed to stand overnight. The defattened material was then subjected to four rounds of washing with deionised water, each lasting fifteen minutes, within a shaker incubator set at 125 rpm. Following the washing procedure, the material was rinsed with Triton-X-100, with each round lasting 15 minutes and being performed at room temperature.

Decellularisation was carried out using 0.05% trypsin and 0.02% EDTA in a shaker incubator at 150 rpm for approximately six hours. Two changes of the enzyme solution were performed at three-hour intervals. The decellularised material (Fig.3) was subsequently washed with deionised water for 30 minutes to remove excess enzyme. Cellular material that was extruded after decellularisation was removed from the scaffold by washing it with a 1% Triton-X-100 solution for two hours at 37°C in a shaker incubator. Excess detergent was then removed by washing the scaffold with deionised water four times, each wash lasting about 30 minutes. The decellularised scaffold was subsequently dried in laminar airflow overnight and sterilised by gamma irradiation in a gamma irradiation chamber at a dose rate of 25 kGy.

Tunica vaginalis upon decellularisation got transformed into a thin, transparent, tough and unique acellular scaffold. The confirmation of the absence of nuclear material in the decellularised scaffold was conducted through histological evaluation (Fig.4-5). On H&E staining, the non-decellularised tunica vaginalis possesses bluish-coloured nuclear remnants and pink-stained wavy collagen while the decellularised material lacks nuclear remnants.

Decellularisation, involved the removal of native cells from tissue, leaving behind a three-dimensional (3D) ultrastructure of extracellular matrix (ECM) proteins while preserving the tissue's bioactivity and mechanical properties (Gupta *et al*., 2017). Various methods, including chemical, physical, enzymatic, and sometimes combinations of these approaches, were employed for the decellularisation process. Here, a combination of chemical and enzymatic process involving trypsin and Titon X 100 has been used. Trypsin was capable of breaking the peptide bonds on the carboxyl-side of arginine and lysine, while Triton-X-100, a non-ionic detergent, gently interfered with the interactions between lipids and proteins, aiding in removal of nuclear materials (Gupta *et al*., 2017).

Before employing decellularised extracellular matrix (ECM) as a medical device or implant, it's essential to subject it to sterilisation procedures. Extracellular matrix was a biologic substance that required special considerations for sterilisation, including the shrinking temperature of collagen, negative effects on any bioactive components, changes in ultrastructure, and changes in surface properties (Badylak *et al*., 2008). Here we used gamma irradiation at a 25 kGy dose rate for sterilisation, as proposed by Thajunnisa *et al.* (2020) and Megha *et al.* (2022) for sterilisation of bovine omental scaffold.

Conclusion

Usage of decellularised bovine

Fig.1 Bovine testis

Fig.2 Tunica vaginalis

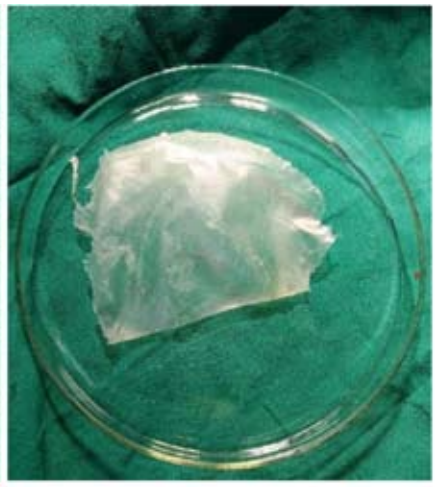

Fig.3 Decellularised tunica vaginalis

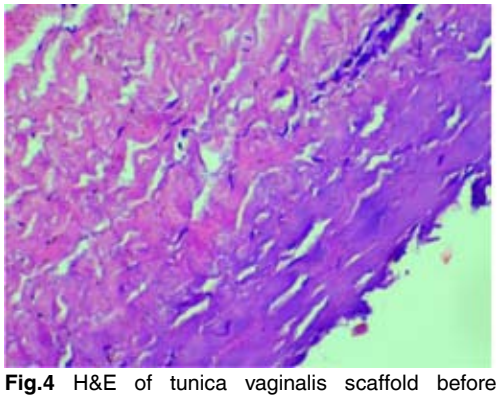

Fig.4 H&E of tunica vaginalis scaffold before decellularisation with nuclear remnants

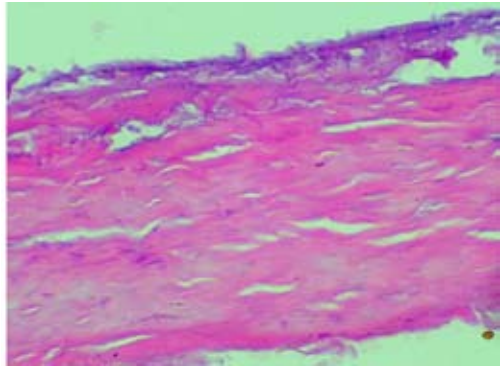

Fig.5 H&E of tunica vaginalis scaffold after decellularisation without nuclear remnants

tunica vaginalis collagen membrane as a tissue engineered scaffold was a novel approach to tissue engineering. Thin, strong and flexible collagen scaffold was obtained by decellularising bovine tunica vaginalis by combination of enzymatic and chemical methods using the Trypsin-EDTA protocol with Triton X-100 as a detergent.

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Conflict of interest

The authors declare that they have no conflict of interest.

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