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Original article

Hemocompatibility and surface properties of bovine pericardial patches: Effects of gamma sterilization

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ABSTRACT

In the present study, the effects of sterilization by gamma irradiation on hemocompatibility and surface properties of the acellular bovine pericardium, a versatile biomaterial were investigated. The native bovine pericardium was decellularized using SDS for one group, Triton-X 100 for other group and subsequently sterilized with gamma rays. The chemical composition was confirmed using infrared spectroscopy, morphological changes by scanning electron microscopy and in vitro hemo-compatibility studies were conducted. Our results indicated that gamma ray sterilization process had significant effects on the processed bovine pericardium. A confirmed decrease in hemocompatibiity, with changes in the collagen organization and fragmentation was noticed. Conventional sterilization methods are not suitable for the targeted biomaterial since the proteins in biomaterial get denatured, compromise their physicochemical properties and hemocompatibiity.

1. Introduction

Biological scaffolds composed of extracellular matrices (ECM) are commonly used for a variety of reconstructive surgical applications and are increasingly used in regenerative medicine strategies.[1] Xenogeneic and allogeneic ECM have been used as bioscaffolds for the reconstruction of many different tissue types in human clinical studies.[2] The bovine pericardium (Bp) is used especially in cardiovascular surgery for repair of atrial septal defect, ventricular septal defect, soft tissue repair, and strengthening the suture line during general surgical procedures etc.[3] Bovine pericardial patches have several benefits over synthetic prosthetic patches [e.g. polytetrafluoroethylene, polyethylene terephthalate (Dacron®)], which include functional host tissue integration, greater biocompatibility, decreased suture line bleeding, ease of maneuvering and reduction of infection rates.[2,4]

Sterilization is the most important step in manufacturing sterile biomedical devices intended for clinical use as an implant. The worldwide accepted definition of sterility is defined as the chance of finding a viable organism in or on a medical device at the most one in a million.[5] Ethylene oxide sterilization requires exposing the tissue to increased temperatures and vapor would induce oxidative damage in the tissue.[5] Electron beam irradiation will also cleave the collagen backbone and lead to deterioration of the tissue structure.[6] Gamma ray sterilization has excellent penetration vis-a-vis UV radiation or X-ray beam; moreover the absolute reliability and efficiency with a stipulated dose for sterilization is also well known apart from its cost effectiveness. It does not require residue removal as is required in case of ethylene oxide sterilization.[7] In gamma ray sterilization, the radiation delivers a certain dose that takes time from minutes to hours to penetrate and produce the effect, depending on the thickness and the volume of the product. Gamma irradiation at a dose of 25 kGy or 2.5 Mrad used in this present study, kills all bacteria, fungi, viruses and spores.

Gamma rays are known to generate significant reactive oxygen species in collagenous substrates which cause backbone scission and breakage of collagen. Furthermore the structural and biological properties of biomaterials can be affected by gamma irradiation; it can induce cross linking in polymers and may cause structural changes.[8,9] This damage would lead to decreased mechanical and biochemical functionality in the tissue. Biocompatibility and bioactivity of biomaterials are known to be affected post irradiation, and it was previously demonstrated that the bioconductivity and absorption of biomaterials are dependent

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upon irradiation dose.[6] Methods such as liquid chemical sterilization and aseptic processing may be chosen for sterilization processes of biomaterials in order to preserve the desired properties of the tissue after sterilization.[10] Previously, we have analyzed the effects of gamma irradiation on mechanical features of processed biomaterials.[11] Thrombogenicity and haemolytic index of biomaterials are important when the material is designed for use in the vascular system where it is in direct contact with blood. However, there are very few studies which simultaneously assessed the impact of gamma radiation or chemical sterilization approaches on hemocompatibiity of acellular bovine pericardial patches.

The Bp suitable for clinical use, requires chemical and physical pre-treatment aimed at removing the immunogenicity of the material, strengthening and sterilizing the tissue and subsequently preserving the tissue for a better shelf life.[10] The Bp was chosen to be studied in the present work because it is a versatile biomaterial used in multiple surgical applications.[12] The objectives of the present study were (1) to investigate the surface topography and collagen fibril organization of Bp before and after gamma ray sterilization; (2) to compare microbiological load and hemocompatibiity of chemically sterilized and gamma ray sterilized Bp.

2. Materials and methods

2.1. Preparation of acellular bovine pericardium

The Bp from adult buffalo was harvested directly from an inspected abattoir and transported within 48 h to the laboratory. The adherent fat was carefully removed, and the pericardium was cut into pieces of 5 cm × 5 cm.

The treated bovine pericardia were categorized as:

- Group I (SDS; $n = 10$): the Bp treated with 0.5% SDS – an anionic detergent.
- Group II (TX; $n = 10$): the Bp treated with 0.9% Triton X-100 – a non-ionic detergent.

The decellularized Bp were further treated with 0.6% glutaraldehyde (GLUT) in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered saline (pH 7.4) for 5 days. The treated bovine pericardium was stored at 4 °C in sterile HEPES buffer.[4]

2.2. Histological analysis

Tissues of all the groups were fixed with 10% phosphate-buffered formalin and embedded in paraffin. The cut sections were stained with hematoxylin and eosin (H&E) for evaluation of cell removal.

2.3. Sterility test

The pericardial patches were aseptically transferred into fluid thioglycollate and soyabean casein digest sterile culture media and incubated for 14 days. Clouding of the broth indicated contamination and inefficient sterilization. Any turbidity in the tubes was followed up by Gram's staining and subculture on appropriate culture medium.

2.4. Gamma irradiation

The samples from SDS and TX treated were exposed to gamma irradiation at doses of 25 kGy at a rate of 3.93 kGy/h using cobalt-60 as a radiation source.

2.5. Fourier transform infrared spectroscopy (FTIR)

Analyses were performed on non-irradiated and irradiated bovine pericardial patches. The moisture content in Bp was removed using ethanol as a dehydrating agent. The samples were immersed in an ethanol solution of different concentrations in series i.e. 75%, 85%, 95%, 100% for a period of at least 15 min in each. Functional groups present in scaffolds were identified using FTIR in the range of 400–4000 cm^{-1} at a resolution of 8 cm^{-1}.

2.6. Scanning electron microscopy

For scanning electron microscopic (SEM) examination, cross-linked Bp were dehydrated using graded alcohol and examined under a field emission scanning electron microscope (FEI-QUANTA 200F, Eindhoven, Netherlands) at an accelerating voltage of 10 kV.

2.7. Complete clotting time measurement

The thrombo-resistant properties of the Bp were evaluated using the clotting time method ($n = 10$). 0.1 ml of fresh fish blood was dropped onto the glass slide and after a pre-determined time, the samples were transferred into 10 ml of distilled water. The concentration of free hemoglobin in the water was measured by monitoring the absorbance at 540 nm using a spectrophotometer. The absorbance values were plotted versus the blood contacting time.

2.8. Evaluation of hemolytic properties

The samples under examination ($n = 10$) were transferred to individual test tubes, and 1.0 ml of fish blood was added. The samples were incubated at 37 °C for 3 h and centrifuged at 800 × g for 15 min. The supernatant was analyzed for hemoglobin concentration and percentage of hemolysis was calculated. Water for injection was used as a positive control; where as high-density polyethene was used as a negative control.

2.9. Statistical analysis

The experiments were conducted at least in triplicate and averages were presented as mean ± standard deviation (SD). Differences were determined by using the paired t-test and a p-value of <0.05 was considered statistically significant.

3. Results and discussion

Recent advancements in tissue engineering and regenerative medicine have led to the development of biomaterials for cardiac reconstruction, repair of a hernia, breast reconstruction, and other soft tissue applications. A sterilization process is essential for every material or device for clinical use and gamma irradiation has frequently been used for sterilization of biomedical implants. However, structural and biological properties of materials could be affected by gamma irradiation.[13] Higher doses of gamma irradiation would induce cross-linking in polymers, and considerable structural changes might occur after irradiation.[14] Biocompatibility and bioactivity of biomaterials could also be affected, and it was shown that the conductivity and absorption of these materials were dependent upon irradiation dose.[13,15] The aforementioned changes in the properties of biomaterials after sterilization by gamma rays may affect their applications in tissue engineering.

Bovine pericardial patches have been mostly used for cardiovascular applications, i.e. vascular grafts and heart valves. Additionally, Bp has also been used for the construction of