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THE EFFECT OF PRESERVATION AND IRRADIATION ON TENSILE STRENGTH OF BOVINE PERICARDIUM

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SUMMARY

Bovine Pericardium (BP) bioprosthesis has been in clinical use for over a decade. In all conscience, bovine pericardium always works as cost effectiveness bioprosthesis because the materials are obtained from organs and animal tissue rejected by sloughterhouse. Use of different chemical or physical procedures to improve the resistance of the graft to enzymatic or chemical degradation, to reduce the immunogenicity of the graft and to sterilize and sustain the graft mechanical integrity and natural compliance. Freeze-drying BP is an useful technique of preservation with significant effect on the tensile strength for surgical application.

1.0 Introduction

BP is composed of outer layer, the fibrous pericardium, and an inner layer, the serous pericardium1-2. It has been widely used in reconstructive surgery for fabrication of heart valve, thoracoabdominal wall defect surgery, barrier material for periodontal guided tissue regeneration and as dural grafts3-9. It acts as a bridge for migration of fibrotic tissue ie., as a matrix for the deposit of collagen and thus enhances formation of fibrous tissue scar on the prosthesis and surrounding tissue 5-7. Procedures that normally used for bovine pericardium preservation and sterilization are chemical treatment with, lyophilization (freeze-dried), glycerolization and gamma irradiation4-9. Glycerolization has been proven to be relatively simple, inexpensive procedure and can be stored up to 5 years (Zuki et al., 2007). Freeze-dried can be solely used for preservation of bioprosthesis or in combination with gamma irradiation prior to surgical application (Zuki et al., 2007). The advantage of using physical methods instead of chemical methods is that they do not introduce chemicals that may cause potential harm for reconstruction of surgical defects or as tissue patches. This study was aimed to identify changes in biophysical properties of two different preservation technique namely freeze-dried and glycerol preservation and effect after gamma radiation.

2.0 Materials and methods

Each different chemical or physical procedure used a total of 80 sample tissue. Then, they were divided into two equal groups, half of the sample were sent gamma irradiation at 25 kGy at Malaysian Nuclear Agency.

2.1 Fresh Bovine Pericardium (FBP)

Pericardial sacs were collected from healthy adult cattle from slaughterhouse. After removal of the adherent fat, the pericardium was washed thoroughly under running water for injection (WFI) and cut into 1cm × 4cm size.

2.2 Freeze Dried Bovine Pericardium (FDBP)

The pericardium were soaked in 0.05% sodium hypochlorite for 2 hours at room temperature before being cleaned with sterile WFI by using multi wrist shaker for 3 times for 15 minutes each washing cycle. The pericardium was placed in deep freezer at -30oC for 4 hours. Then, the pericardium was placed in freeze dryer for 72 hours. The FDBP then sealed in triple layer polyethylene bag and stored at room temperature.

2.3 Glycerolized Bovine Pericardium (GlyBP)

Pericardium soaked in 0.05% sodium hypochlorite for 2 hours at room temperature before being clean with sterile WFI by using multiwrist shaker for 3 times for 15 minutes each washing cycle and then cut into 1cm × 4cm size. The pericardium placed into 50% sterile glycerol in sterile normal saline and kept at room temperature for 3 hours. Then, they were transferred into 70% sterile glycerol in sterile normal saline and kept at room temperature for another 3 hours. Finally, transfer the pericardium into 80% sterile glycerol in a sterile bottle with tight cap and kept at room temperature for before stored in refrigerator at 4oC.

2.4 Testing and analysis:

The sample were loaded to the instron grips by pneumatic clamp, 3 cm of the strip length was exposed between the instron grips and the remaining 1cm was clamped between the upper and lower clamping devices. Tensile test was done by using computer controlled instron tensiometer machine (Instron 430l) equipped with 500N cell which determined tensile stress under varying rates of strain or elongation.

3.0 Results

One-way ANOVA test shows that there was a significant difference in tensile strength between the six groups with $p < 0.001$ and F-statistic (5,234) = 22.24

Post-hoc comparison (Table 1) using Dunnett T3 shows mean tensile strength was significantly different for only seven comparisons. All seven comparisons have significant p-value ($p < 0.001$). The comparison was FBPNR and FDBPNR, FBPNR and FDBPIRR, FBPIRR and FDBPNR, FBPIRR and FDBPIRR, FDBPNR and GBPNR, FDBPNR and GBPIRR, and, FDBPIRR and GBPIRR.

Table 1 Post-hoc analysis by Dunnett T3 for the comparison of mean tensile strength among six groups

Comparison		*Mean difference (95% CI)*	*p-value*
FBPNR	FBPIRR	0.93 (-6.78, 8.65)	> 0.95
	FDBPNR	-20.02 (-27.76, -12.28)	< 0.001
	FDBPIRR	-13.97 (-21.09, -6.86)	< 0.001
	GBPNR	-5.78 (-13.19, 1.63)	0.263
	GBPIRR	0.85 (-4.74, 6.44)	> 0.95
FBPIRR	FDBPNR	-20.95 (-30.27, -11.64)	< 0.001
	FDBPIRR	-14.91 (-23.74, -6.08)	< 0.001
	GBPNR	-6.71 (-15.77, 2.35)	0.339
	GBPIRR	-0.08 (-7.81, 7.65)	> 0.95
FDBPNR	FDBPIRR	6.05 (-2.80, 14.90)	0.464
	GBPNR	14.24 (5.17, 23.32)	< 0.001
	GBPIRR	20.87 (13.12, 28.62)	< 0.001
FDBPIRR	GBPNR	8.19 (-0.38, 16.77)	0.072
	GBPIRR	14.82 (7.69, 21.95)	< 0.001
GBPNR	GBPIRR	6.63 (-0.79, 14.05)	0.119

**(n = 240). One-way ANOVA test was applied followed by post-hoc comparison using Dunnett T3 test.*
F-stat (5, 234) =22.24, p < 0.001

4.0 Discussion

This study demonstrates tensile strength of freeze dried bovine pericardium (FDBP) is better compared to glycerolized bovine pericardium (GlyBP) preparation. Hence, this finding could greatly influence in choice of preservation of bovine pericardium as bioprosthesis in clinical usage in future.

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