Development of in vitro test system to assess hyperacute immune response to decellularized bovine pericardium using human donor blood

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Introduction
We previously showed that tissue decellularization by irradiation of microwave under pulsatile detergent circulation realized cell removal while preserving biomechanical properties1). It is well recognized that transplantation of porcine or bovine xeno-tissues into primate rapidly causes hyperacute immune-rejection within minutes or hours. Safety of decellularized xenogeneic tissues, therefore, remains great concern. The aim of this study is to develop an in-vitro test system to assess hyperacute immune response to decellularized bovine pericardium using human donor blood.

Materials and Methods
1. Test system and procedure description
This study was approved by ethical committee of Waseda University. Venous blood was drawn from healthy volunteers, with 200 unit of heparin to 60cc of blood, and 75mg of glucose was added. A chamber which composed of blood supply and sampling ports and gas-permeable silicone tube was manufactured. The chamber was incubated in 2% CO2 and 37°C. The chamber was continuously rotated at the speed of 40 rpm in order to prevent separation of blood cells and plasma components. Blood samples were collected at start, in one hour, 3 hours, and 5 hours. Blood pH and glucose were examined.

2. Decellularized bovine pericardium
Bovine pericardium with a thickness of approximately 0.4mm were decellularized by microwave irradiation for 24 hours and pulsatile flow-and-pressure circulation of 1 wt% deoxycholic acid (Sigma). Then the tissues were treated with 100 U/ml Benzonase® endonuclease (Novagen) in continuous shaking condition at 37°C for 24 hours. Decellularized pericardium were freeze-dried and sterilized by gamma-radiation of 35kGy. Prior to tests, the tissues were re-hydrated with phosphate buffered saline (PBS, Invitrogen).

3. Assessment of hyperacute immune response
Decellularized and sterilized bovine pericardium were incubated with human blood for 5 hours. Non-treated bovine pericardium washed with PBS was also tested as control. Frozen tissue sections were stained using DAPI (4’,6-Diamidino-2-phenylindole dihydrochloride, Sigma) and C5b-C9 terminal complement complex(DAKO), which is one of representatives of complement activation.

Results and Discussion
Throughout 5hours’ test period, pH was maintained within normal ranges of 7.31~7.45. Glucose was consumed with time in 5hours, and the final concentration at termination was kept within normal ranges. Fluorescent immunostaining showed heavy deposition of C5b-C9 in non-treated bovine pericardium (Fig2(a)). However, for the decellularized and sterilized bovine pericardium, deposition of C5b-C9 was markedly suppressed (Fig2(b)). The data suggested that decellularized and gamma-radiation sterilized bovine pericardium distinctly reduced complement activation.

Conclusions
The test system which can maintain blood pH within physiologic ranges was successfully developed. The data suggested that the system work for the assessment of hyperacute immune response in vitro.

Reference

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