Regenerative Tissue Scaffolds Prepared by Gamma Ray Irradiation

Toshia Fujisato[1], Seiichi Funamoto[2], Ken'ichi Yoshida[3], Tetsuji Yamaoka[1], Tsuyoshi Kimura[2], Masahiro Kikuchi[4], Yasuhiko Kobayashi[4], Akio Kishida[2] and Takeshi Nakatani[1]

[1]National Cardiovascular Center, Osaka, Japan
[2]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan
[3]Foundation for Biomedical Research and Innovation, Kobe, Japan
[4]Takasaki Advanced Radiation Research Institute, Japan Atomic Energy Agency, Takasaki, Japan

Statement of Purpose: The reconstruction of heart valves using acellular xenogeneic scaffolds has been studied to have more durability with growth potential applicable to pediatric patients. Most of the groups developing acellular scaffolds have been using detergents and/or enzymes as decellularization media such as Triton® X-100, sodium dodecyl sulfate, deoxy-cholate, trypsine, DNase, and RNase. Since the detergents are generally cytotoxic and it takes time for their removal before the transplantation, it may lead denature of biological properties and contamination in the process. We have been developing several tissue processes for preparation of acellular grafts using ultrahigh pressure, microwave irradiation, and supercritical fluid extraction. In this paper, a novel process using γ-ray irradiation has been reported. All of these processes do not include any detergent and may be applicable to relatively large tissues.

Methods: Mouse, rat, and porcine vascular tissues were isolated and irradiated by the γ-ray of 10, 30, 100, 300, and 1000 Gy in PBS at room temperature. The dosage rates were 100, 300, 100, 300, and 1000 Gy/hr, respectively. They were then rinsed by PBS-based washing solution including DNase and RNase at 4 °C for 1 week. The tissues treated were subjected to histological study, residual DNA assay, and biomechanical study by the tensile strength measurement.

The acellular porcine aortas were implanted in subcutaneous space of Wister rats. The grafts were explanted and examined histologically after 2 weeks of implantation. All animals were carefully reared in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication No.85-23, revised in 1985).

Results / Discussion

There were no cells observed in the tissues pretreated by the γ-ray more than 300 Gy (Fig.1). The amount of DNA in the tissue was lower than 10% of that in the native tissue (Fig.2). There were no significant changes in biomechanical properties of breaking strength and elastic modulus in the acellular tissues.

There were mild tissue responses of T-calls and macrophages observed in the acelluar tissue prepared by γ-ray irradiation of 1000 Gy whereas severe responses observed in the control tissue 2 weeks after the implantation to rat subcutaneous space (Fig.3).

Conclusions:

This process may have more secure acellular scaffolds for the tissue regeneration.

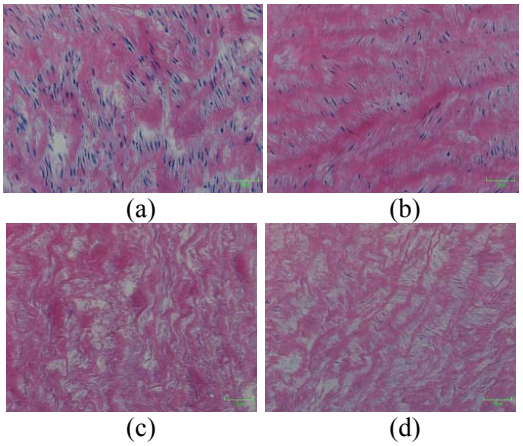

Fig.1 The (a) native and γ-ray pretreated porcine aortas of (b) 30, (c) 300, and (d) 1000 Gy followed by washing.

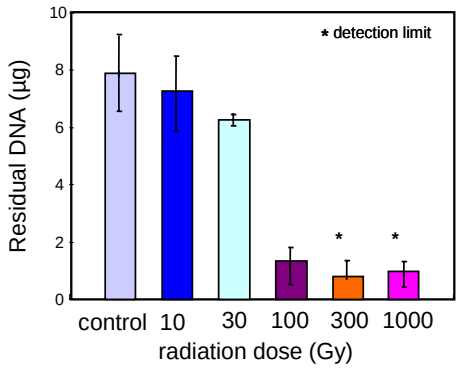

Fig.2 The amount of residual DNA in the treated aorta.

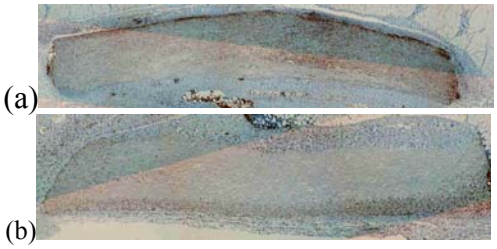

Fig.3 Anti-CD68 (macrophage) staining of (a) native and (b) acellular porcine tissues 2 weeks after implantation to rat subcutaneous space.

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