

Calorimetric study of extracellular tissue matrix degradation and instability after gamma irradiation

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Abstract

Native extracellular tissue matrix (ECM) is increasingly used for tissue repair and regeneration. The kinetics of gamma irradiation damage on human dermis ECM was studied by differential scanning calorimetry (DSC). Dermis ECM was irradiated at a low-dose rate of 0.23 kGy h^{-1} in order to study the progression of ECM damage as the gamma dose increased from 0 to 32 kGy. The study showed that the effect of gamma irradiation above 2 kGy was predominantly peptide chain scission. As the gamma dose increased, the stability of irradiated ECM decreased further, and multiple ECM domains of different stability were detected. Even a moderate gamma dose (7–12 kGy) could decrease the onset denaturation temperature of ECM to below body temperature. DSC analysis also showed partial and spontaneous protein denaturation in gamma-irradiated, rehydrated ECM at 37 °C. In vitro rehydration tests confirmed that a significant fraction of the irradiated ECM disintegrated into minute ECM fragments at 37 °C, although the irradiated ECM appeared to be normal at 4 °C and room temperature. DSC data were correlated well to effects of gamma irradiation on ECM microstructure, mechanical property and in vitro cell response reported earlier by us. A model was presented to describe the kinetics of gamma-irradiation-induced alterations of tissue ECM properties.

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1. Introduction

Decellularized tissues and organs of animal and human origins provide an excellent 3-D scaffold architecture and biological milieu for tissue engineering and in situ tissue regeneration in human patients. Decellularization yields a complex structured composition of functional proteins (ECM), which supports cell in-growth and re-vascularization. In recent years, various ECM materials have been successfully used in a variety of applications including replacement and/or repair of diseased or damaged skin, heart valve, blood vessel, tendon, ligament and abdominal wall [1–7]. The ECM serves as a template for tissue repair, and its use may eliminate pathological complications associated with the use of many alloplastic materials [1,8–13].

The important factors that contribute to successful clinical applications of ECM materials are their bio-inductive properties, good biomechanical properties, the ability of remodeling, and the desirable host tissue response to naturally occurring ECM [5,6].

One of the significant challenges is to ensure the sterility of ECM products without damaging their structural integrity and compromising their clinical performance. Ionizing irradiation (e.g., e-beam and gamma) is widely used as a terminal sterilization method for biomedical devices. However, ionizing irradiation severely damages tissue ECM and other collagen-based materials even at an irradiation dose that is much lower than the dose required for sterilization [14–16]. Such damage on collagen-based materials has raised serious concern on the clinical use of irradiated tissue grafts such as patellar tendons [17–24], although successful use of some irradiated human tissues in patients was well documented [25–28].

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A survey of research literatures has identified significant knowledge gaps in our current understanding about the effect of ionizing radiation on collagen-based materials. At present, tissue damage by ionizing irradiation is interpreted in terms of molecular fragmentation and degradation via direct peptide chain scission, as well as free-radicals-mediated cross-linking, both of which could well affect bio-inductive properties and biomechanical properties of tissue grafts and the ECM-based products. This theory is based on the results of a number of studies done between 1950 and 1973 on irradiated collagen materials [29–42]. These earlier studies investigated the effects of irradiation doses from 200 kGy to 2200 kGy, and occasionally tested lower irradiation doses at 50–100 kGy. Irradiation damage on collagen materials at the dose range tested was found to be resulted mainly from peptide chain scission when irradiated in dry state, and from intermolecular cross-linking when irradiated in hydrated state. But the dose of e-beam and gamma-irradiation for sterilization of tissue grafts and collagen-based products is in the range of 20–40 kGy [14,15,18–24,43–45], and therefore, a reasonable dose range for studying the mechanisms of ionizing irradiation damage should be 0–40 kGy. The conclusions from those earlier studies and the theory derived from them probably described a different type of irradiation damage on collagen and tissues, which may not be relevant to the development of sterilization methods for tissue grafts and ECM-based products.

More recent studies (1989 to present) investigated the irradiation-induced changes at the dose range related to sterilization of collagen-based products. These studies were conducted on diverse collagen systems, ranging from soluble collagen suspensions, cross-linked collagen gels or solid matrices, acellular ECMs (processed dermis and small intestine submucosa) to cellular tissue grafts (heart valve, meniscus and tendon). Ionizing irradiation at the sterilization dose or lower dose range was observed to affect collagen matrix microstructures [14,16,44,46], biomechanical properties [17–24,43,45,47], biocompatibility [10,44,48–50], and the stability against enzyme degradation and thermal denaturation [14,15,19,44,45,50]. Collectively, these studies have demonstrated that the extent of irradiation effect is dependent on the irradiation dose, condition (atmosphere and temperature), collagen type, hydration, chemical modifications and processing methods. However, individual studies often examined one particular type of collagen materials at a limited irradiation (e-beam or gamma) dose range, and used different metrics to assess irradiation damage on the products. It was difficult to compare the data from one study to another, even for the same type of collagen materials and similar irradiation methods or conditions. Besides a few studies on free-radicals reactions upon ionizing irradiation [52–54], none of the recent studies was aimed to elucidate molecular mechanisms of irradiation damage on collagen-based materials. Without a better understanding of molecular mechanisms of irradiation damage, it is not known how, as dose increases, ion-

ization irradiation alters ECM material properties. In addition to sterilization, low-dose gamma-irradiation has been introduced as a processing method to modify certain product properties [16,48,49,51]. Understanding molecular mechanisms of irradiation damage could provide a scientific rationale to guide the process development.

The objective of the present study was to investigate the kinetics of gamma-irradiation damage on tissue ECM as the irradiation dose increased gradually from 2 to 32 kGy. Tissue ECM used in the study was commercially available, decellularized human dermis (AlloDerm, LifeCell Corporation, Branchburg, NJ). Gamma irradiation damage on tissue dermis ECM was examined, using differential scanning calorimetry (DSC) to monitor the change of the thermal stability profile of ECM proteins (mainly type I collagen). DSC is a sensitive method for examining molecular changes in tissue ECM after gamma irradiation, including cross-linking, peptide scission, collagen conformation, triple helical content, and fibrillogenesis of collagen molecules in ECM [55–62].

2. Materials and methods

2.1. Preparation of human acellular dermal matrix (ECM)

Human acellular dermal matrix was produced using donated cadaver skin tissue with the consent for research use. Donated skin tissue was processed by removing epidermis and cellular components while retaining major biochemical components and their extracellular structural integrity. Details of the processing method and freeze-drying preservation were described elsewhere [1,63,64].

Major biochemical components in the ECM included collagen, elastin, proteoglycans, and tissue lipids, as well as carbohydrates that were used as the lyoprotectant during freeze-drying. Lipid-free and carbohydrate-free tissue matrix was also made for comparative studies, which assessed the effects of tissue lipids and carbohydrates on the resistance of ECM to gamma irradiation. Donated tissue was processed using the same method, but processed tissue was freeze-dried without the lyoprotectant solution. Then lipids in freeze-dried tissue matrix were removed by direct chloroform extraction without tissue rehydration (solid phase extraction). Freeze-dried porous ECM was submerged in 100% chloroform (30 ml per g dry tissue) for 48 h with chloroform changed after first 24 h extraction. Chloroform residue in tissue matrix was removed by vacuum before gamma irradiation.

Prepared dermal tissue samples had a thickness of $\sim 1.2 \pm 0.2$ mm (mean \pm standard deviation). They were shelf-stable with a residual moisture about 2%, sealed in dry nitrogen and stored at 2–8 °C before gamma irradiation.

2.2. Gamma irradiation treatment

Gamma irradiation experiments were conducted with a GC 4000A cylindrical gamma irradiation chamber

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