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# Physicochemical study of ascorbic acid 2-glucoside loaded hyaluronic acid dissolving microneedles irradiated by electron beam and gamma ray

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ABSTRACT

A dissolving microneedle (DMN) patch encapsulated with ascorbic acid 2-glucoside (AA2G) in a needle-shaped hyaluronic acid (HA) backbone was fabricated and sterilized by electron beam (e-beam, 5–40 kGy) and gamma ray (γ-ray, 5–30 kGy). DMN structures maintained their morphologies and fracture force regardless of e-beam and γ-ray irradiation doses. Both e-beam (40 kGy) and γ-ray (20 and 30 kGy) met the product sterility requirements for cosmetics and vaccines; however, γ-ray irradiation significantly degraded the encapsulated AA2G, while e-beam maintained AA2G activity. Thus, an e-beam dose of 40 kGy, which satisfied the sterility requirements without loss of AA2G, is suitable for terminal sterilization of DMNs. Moreover, we confirmed that the optimized irradiation (e-beam, 40 kGy) did not affect dissolution rate and drug release profile of DMNs. Further, we confirmed that HA, the backbone polymer of DMNs, could be utilized as a stabilizer that inhibits degradation of encapsulated AA2G by irradiation. This detailed analysis can be developed further to optimize various biological drugs in transdermal drug delivery systems.

## 1. Introduction

Drugs such as biotherapeutics or vaccines are typically administered by injection, which is a low-cost, rapid, and direct way to deliver almost any type of molecule. However, hypodermic needles cannot easily be used by patients at home, and can cause various side effects such as skin irritation, pain, and local injury, leading to discomfort (Giudice & Campbell, 2006; Rogers, 2010). Although oral delivery usually prevents these problems, many drugs cannot be administered orally because of low absorption rates and drug degradation in the gastrointestinal tract (Rowland, 1972; Singh, Singh, & Lillard, 2008). To overcome these limitations, numerous transdermal drug-delivery systems have been developed, combining enhanced efficacy with patient-friendly delivery (Anirudhan, Nair, & Nair, 2016; Kong & Park, 2011; Kong, Kim, & Park, 2016; Prausnitz & Langer, 2008).

Dissolving microneedles (DMNs) have been conceptualized and developed as one such drug-delivery system (Kim, Park, & Prausnitz, 2012; Park, Allen, & Prausnitz, 2005; Tadwee, Gore, & Giradkar, 2011). Drugs are encapsulated in a needle-shaped biodegradable polymer matrix and released directly into the dermal layers as the polymer needles penetrate the skin; this method enhances drug delivery while improving patient compliance and safety (Chu, Choi, & Prausnitz, 2010; Kim, Kim, Yang, Lee, & Jung, 2013; Kim et al., 2016; Mistilis, Bommarius, & Prausnitz, 2015). However, as DMNs pierce the skin and contact tissues directly, the final product must meet stringent standards of sterilization for application in patients. A failure of product sterility could lead to an infection from microorganisms, and such a product should not be released in the market (Tidswell, 2011). To meet standards of product sterility, either terminal sterilization or manufacture via aseptic processing is necessary. Terminal sterilization is the process whereby a product is sterilized in its final packaging, while the aseptic processing sterilizes individual products separately and assembles in a sterile environment. However, aseptic manufacturing can raise the production cost and only be used when terminal sterilization is inapplicable (U.S. Food and Drug Administration, 2004). Therefore, the terminal sterilization process is the most important step in eliminating microorganisms economically and effectively.

Depending on the purpose of sterilization and the material to be sterilized, various terminal sterilization methods have been developed, including dry-heat sterilization, pressured-vapor sterilization, chemical sterilization (with ethylene, formaldehyde, or peracetic acid), and radiation sterilization (electron beam [e-beam] and gamma ray [γ-ray]) (Asasutjarit et al., 2017; Marreco, Moreira, Genari, & Moraes, 2004; Silindir & Ozer, 2009). However, as DMNs are composed of biopolymers

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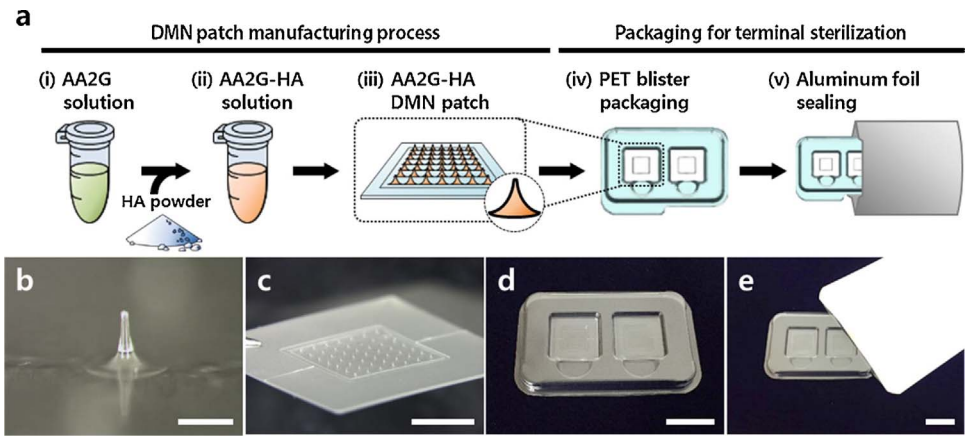


**Fig. 1.** (a) Schematic illustration of overall ascorbic acid 2-glucoside (AA2G)-hyaluronic acid (HA) dissolving microneedle (DMN) patch manufacturing process; (i) AA2G solution, (ii) AA2G-HA solution, (iii) AA2G-HA DMN patch, (iv) PET blister packaging, and (v) aluminum foil-wrapper sealing. (b) Single DMN (scale bar, 300 μm) and (c) whole DMN patch image (scale bar, 5 mm). (d) The patches were placed in PET blister packs (thickness: 280 μm; scale bar, 20 mm), and (e) the blister packs were sealed in aluminum foil wrappers (thickness: 100 μm; scale bar, 200 mm).

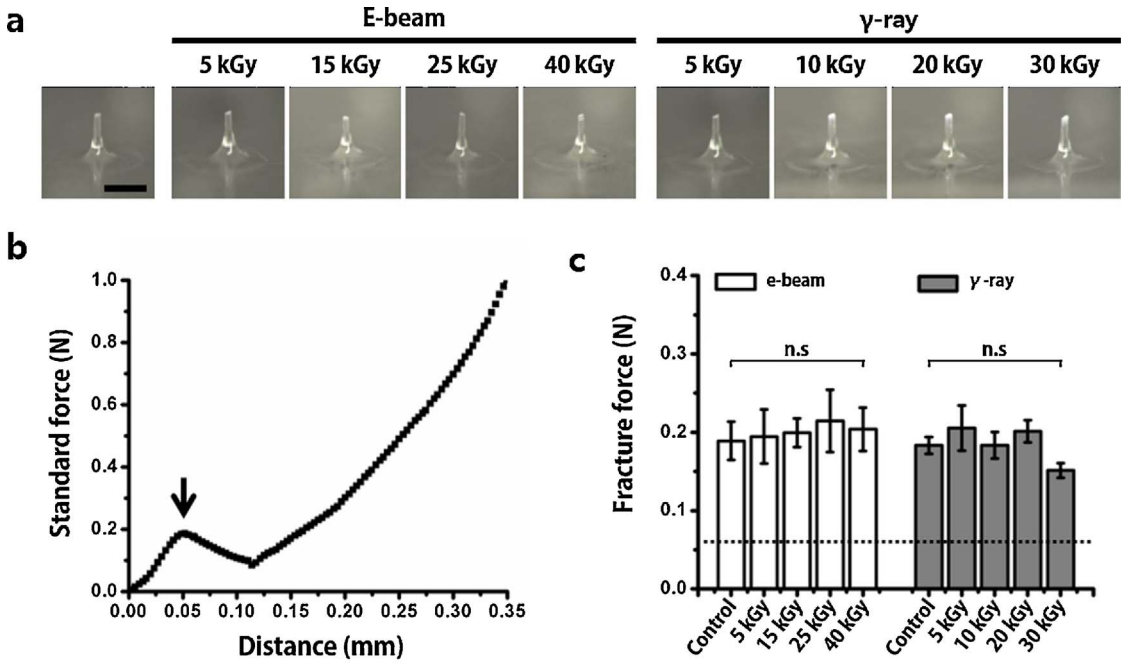


**Fig. 2.** Microscopic images and fracture force analysis of terminal-sterilized ascorbic acid 2-glucoside (AA2G)-hyaluronic acid (HA) dissolving microneedle (DMN) structures. (a) No morphological changes were observed regardless of e-beam and γ-ray irradiation dose (scale bar, 300 μm). (b) Standard force of a single DMN in the control group, recorded by moving the probe as the axial of distance; the peak of the graph indicates the fracture force of a single DMN (arrow). (c) Neither e-beam nor γ-ray irradiation affected the strength of the DMN structures (n = 4, mean ± SEM). The dashed line represents the minimum fracture force of a single DMN required for skin penetration (0.058 N).

**Table 1**
Effects of e-beam and γ-ray irradiation on microbial contamination levels of packaged ascorbic acid 2-glucoside (AA2G)-hyaluronic acid (HA) dissolving microneedle (DMN) patches. AA2G-HA DMN patches were dissolved in 50.0 mL of distilled water and evaluated for total aerobic bacterial viable count, total aerobic yeasts and molds viable count, and the presence of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

| Test item | Control | e-beam (kGy) | | | | γ-ray (kGy) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 15 | 25 | 40 | 5 | 10 | 20 | 30 |
| Total aerobic viable count (bacteria) | < 10 CFU/mL | < 10 CFU/mL | | | | < 10 CFU/mL | | | |
| Total aerobic viable count (yeasts and molds) | < 10 CFU/mL | < 10 CFU/mL | | | | < 10 CFU/mL | | | |
| *Escherichia coli* | None | None | | | | None | | | |
| *Pseudomonas aeruginosa* | None | None | | | | None | | | |
| *Staphylococcus aureus* | None | None | | | | None | | | |

and encapsulated drugs, harsh conditions during the terminal sterilization process, such as heat, moisture, and chemical exposures, can deform DMN morphology and degrade the encapsulated drug, resulting in the failure of DMN functions. Therefore, radiation sterilization, which destroys microorganisms without heat, moisture, or chemicals, is expected to be suitable for DMN product sterilization. However, high-energy transfer from the e-beam and γ-ray irradiations may degrade DMN morphologies or the drug inside the polymer matrix by chemical attacks of free radicals or reactive oxygen species, which can cause dramatically reduced efficacy or induce unexpected side effects (Ameri, Wang, & Maa, 2010; Grieb et al., 2002). Moreover, the degree of this degradation may differ between e-beam and γ-ray irradiations, since γ-ray exhibits higher penetration into sterilizing materials at lower doses than e-beam (Woo & Sandford, 2002). Therefore, selection of an appropriate irradiation method and doses that minimize activity loss of the drug in the DMNs while satisfying standards of sterility is required. To the best of our knowledge, however, an in-depth analysis of these radiation sterilization methods for DMNs has not been reported yet.

In this study, DMN patches composed of hyaluronic acid (HA, with average molecular weight of 39 kDa) and ascorbic acid 2-glucoside