# Effect of gamma radiation dose on the fabrication of alpha-elastin nanoparticles by gamma-ray crosslinking

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**Introduction:**

Elastin is an essential component in animal tissues as well as collagen. Elastin molecules are formed from relatively loose and unstructured polypeptide chains that are covalently cross-linked into a rubberilike elastic meshwork [1]. A unique feature of elastin is that it undergoes self-assembly called coacervation under selected conditions of concentration and temperature called cloudy point (CP).

Taking advantage of this thermosensitive feature, we employed alpha-elastin and examined the formation of nano sized droplets above its CP using various heating procedures. We obtained nanoparticles from the droplets of by gamma-ray crosslinking under a temperature above CP [2].

In this work, we investigated that effect of gamma-irradiation to form crosslinked nanoparticles.

**Materials and Methods:**

***Materials***

Alpha-elastin (extracted from bovine neck ligaments) was purchased from Elastin Product Co., Inc.

***Preparation of alpha-elastin aggregates in solutions by heating***

Ten mg/ml of alpha-elastin dissolved in aqueous solution. The solution of alpha-elastin was slowly heated from 4 $^{o}$C to 60 $^{o}$C in 30 min.

***Dynamic Light Scattering (DLS)***

DLS measurements were done to determine the size of the aggregated alpha-elastin, using a NICOMP MODEL 370 system (USA). The size distributions of the aggregated alpha-elastin before and after irradiation were measured by DLS at 60 $^{o}$C.

***Gamma-irradiation***

The heated alpha-elastin sample was put into thermos bottle and it irradiated at 1, 3, 5, 7.5, 15 and 30 kGy with $^{60}$Co gamma-rays using $^{60}$Co-gamma irradiation pool at Osaka Pref. University (dose rate: 7.9 kGy / h). The temperature of the sample was kept at 60 $^{o}$C during irradiation. We prepared the temperature in the thermos bottle every 1 h.

***Sodium Dodecyl Sulfate-Poly-Acrylamide Gel Electrophoresis (SDS-PAGE)***

In order to analyze molecular weight of the crosslinked polypeptides after gamma-irradiation, we

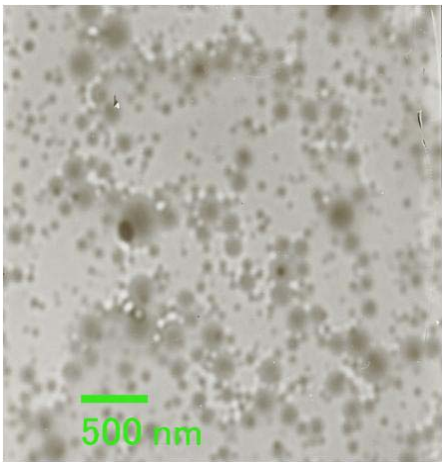
carried out SDS-PAGE. SDS-PAGE was performed using 10 % polyacrylamide gel and 0.5 mg of the polypeptide sample was loaded onto each lane of the gel (Thickness: 1mm) and run in a Mini-PROTEIN 3 cell (Bio-Rad) at constant voltage (200V) for 45 min. Separated polypeptides were visualized by cupper staining kit (Bio-Rad).

***Transmission Electron Microscope (TEM)***

The morphology and particle size distribution of the aggregated polypeptide particles were examined using a HITACHI, EF2000 TEM operated at 200 keV. Samples for the TEM were dissolved in deionized water to a final concentration of 10 mg/ml. The sample solution was added to carbon-coated 400-mesh copper grids (VECO) and excess sample was absorbed in filter paper and the grid was dried at room temperature before observing under TEM.

**Results and Discussion:**

Alpha-elastin was aggregated in aqueous solution with heating to 60oC (above CP) and the size of the aggregates was distributed about 190 nm before irradiation. After irradiation, we did not obtain the stable aggregated alpha-elastin at the doses of 1 and 3 kGy. However after 5, 7.5, 15, and 30-kGy irradiation, aggregated alpha-elastin with the distribution ranging 150 – 220 nm was detected at 60oC (above CP). After cooled to 5oC (below CP), the size of aggregated alpha-elastin were distributed around 60 nm – 70 nm for 7.5 and 15 kGy and 160 nm for 30 kGy, suggesting that we could obtain the stable aggregated alpha-elastin above 7.5 kGy. Yield of the aggregated alpha-elastin collected at 4oC after irradiation was about 10 % irrespective of the dose of 7.5, 15 and 30 kGy. We analyzed the molecular weight of the aggregated alpha-elastin molecules using SDS-PAGE. The molecular weight increased with increasing doses, indicating that the alpha-elastin was crosslinked by gamma irradiation. We observed the morphology of the aggregated alpha-elastin crosslinked by 30 kGy gamma irradiation. Figure showed that almost all of the aggregates were spherical and sizes were distributed within about 60-220 nm at room temperature.




## References:

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