Supporting Information

Increasing stiffness and thermal response behavior of collagen/metal nanoparticles composite hydrogels fabricated through radiochemical reduction

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Contents

Instrumentations and Chemicals

Organo Puric-ω water purification system was used to produce ultrapure water (18.2 Ω cm^{-1}). EYELA FDU-2200 freeze drying system with a 10-station manifold was used for the freeze drying. A JEOL JEM-2100 electron microscope was used to record transmission electron microscopy (TEM) images at an accelerating voltage of 200 kV. A Holey carbon support films coated copper microgrid (EM Japan, U1003) was applied hydrophilic treatment in a glow discharge irradiation chamber before use. Image-J software was used to analyses the TEM images, and the expressed mean diameter and standard deviation are based on an average of 300 particles. Scanning electron microscopy (SEM) images were recorded on a JEOL JSM-6701F field-emission scanning electron microscope at the accelerating voltage of 15 kV. The obtained images were analyzed using JEOL PC-SEM 6701 software. Compression experiments were performed using a SHIMADZU EZ-Test or an Autograph AGS-20NX universal tensile instrument.

Unless otherwise noted, all reagents purchased from commercial suppliers were used without further purification.

Pepsin-treated porcine skin type I and unpurified collagen sponges were donated by NH Foods Ltd.

Phosphate-buffered saline (PBS) powder was purchased from Sigma–Aldrich Japan Inc. and diluted with water (pH = 7.4).

Acetic acid (CH_3CO_2H) was purchased from KISHIDA CHEMICAL Co., Ltd.

Ethanol (99.5%) and silver nitrate ($AgNO_3 \cdot H_2O$) were purchased from Nacalai Tesque, Inc.

Gelatin (077-03155), sodium chloride (NaCl), Tris-HCl buffer, dimethyl sulfoxide, acetone, *n*-hexane, tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), potassium tetrachloroplatinate(II) trihydrate ($K_2PtCl_4 \cdot 3H_2O$), copper(II) chloride ($CuCl_2 \cdot 2H_2O$), and nickel(II) chloride ($NiCl_2 \cdot 6H_2O$) were purchased from FUJIFILM Wako Pure Chemical Corp.

Tetrahydrofuran (THF) and palladium(II) chloride ($PdCl_2$) were purchased from Kanto Chemical Co., Inc.

Hexachloroplatinic(IV) acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$) was purchased by Tanaka Kikinzoku Kogyo Co., Ltd.

Preparation of purified type I collagen solution

1 g of collagen was placed into a 2 L beaker. 500 mL of water was added and stirred at 500–600 rpm in the ice bath. This mixture of collagens was stirred overnight until all the particles were dissolved. To this were added 0.45 mol/L NaCl (13.15 g) and 5 mmol/L tris-HCl buffer solution (394 mg), and the mixture was stirred for 30 min at room temperature. Then, this was transferred to the refrigerator and stored at 4 °C overnight. To this were added 1.2 mol/L NaCl (21.92 g) and 5 mmol/L tris-HCl buffer solution (394 mg), and the mixture was slowly stirred for 30 min at room temperature. Once fully dissolved, it was transferred to the refrigerator and stored at 4 °C overnight. The resulting collagen solution was dispensed into conical tubes (50 mL) and centrifuged for 15 min at 10000 rpm to afford precipitate (The centrifugation process should be repeated when no precipitate was formed). After that, the supernatant was collected into a 2 L beaker, and the solution was transferred into dialysis membranes (15 kDa, length = 15 cm, Spectrum Laboratories, Inc.). This was immersed in water in a 2 L beaker for 7 days. On the first day, the water was changed every hour. From the second day till the seventh day, the water was changed every 3 h to afford a transparent solution. The thus-dialyzed collagen solution was transferred to 50 mL conical tubes, frozen in liquid nitrogen for 30 min, and freeze-dried for 3 days.

The thus-extracted collagen (50 mg) was placed in a 50 mL conical tube. To this was added pre-cooled PBS solution (25 mL), and the mixture was homogenized for 2–3 min until the sponge broke into smaller pieces. It was tightly closed and stored in the refrigerator at 4–5 °C until a homogenous solution was obtained (typically 2–3 days). Then, the solution was centrifuged under 4–5 °C for 5–7 h at 4000 rpm to remove bubbles, giving a 0.2 wt% purified collagen solution. This solution was stored at 4–5 °C.

Pictures of Col-TMNP hydrogels after γ-ray irradiation

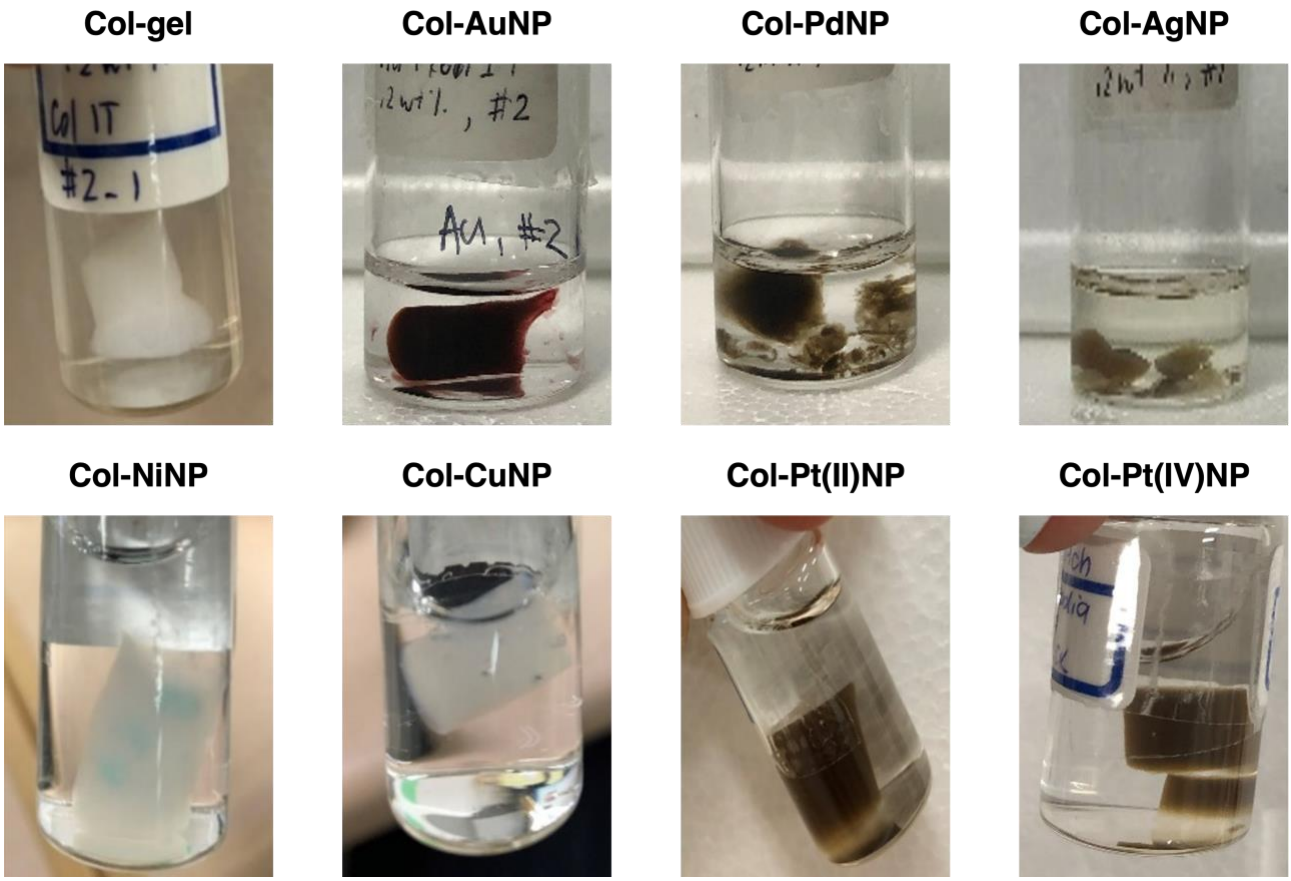

Figure S1. Pictures of Col-TMNP hydrogels after γ-ray irradiation

SEM images of Col-TMNP gels

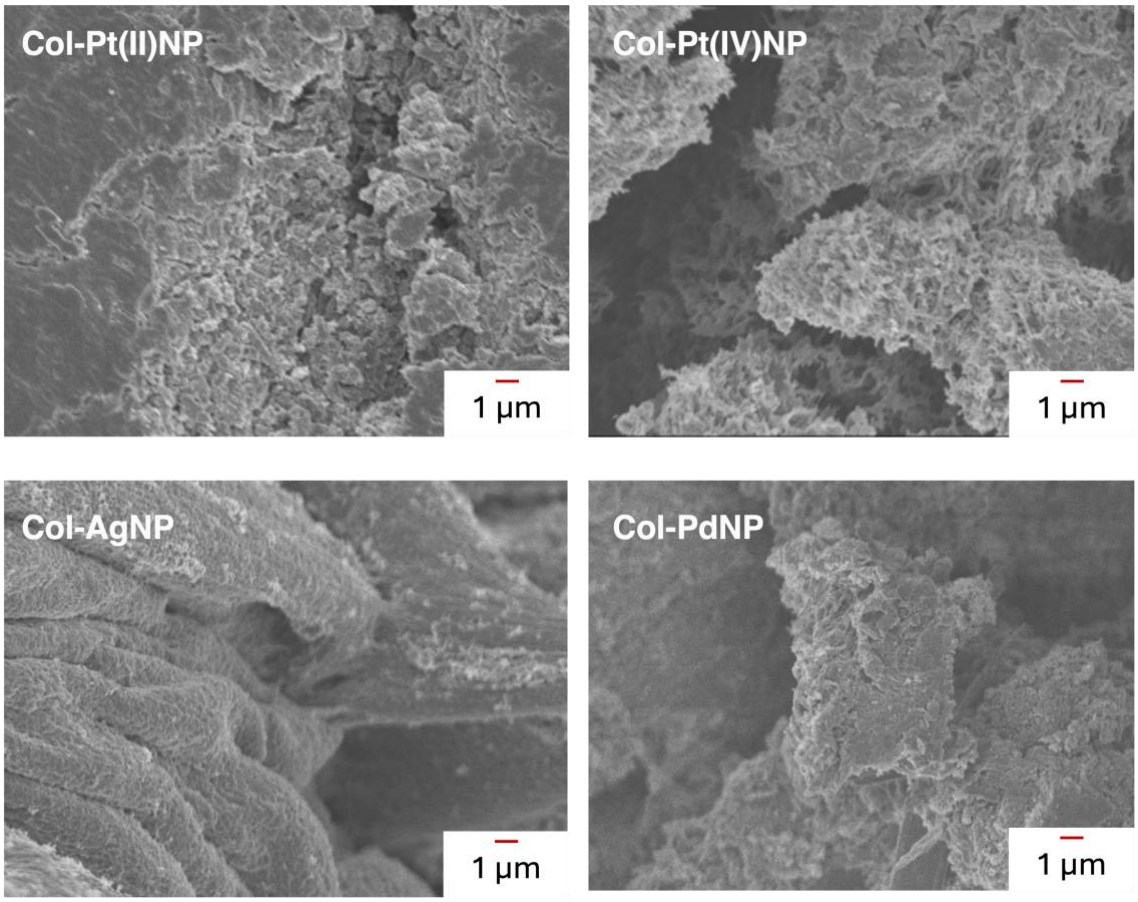

Figure S2. SEM images of Col-TMNP gels

Pictures of metal-gelatin solutions after γ-ray irradiation

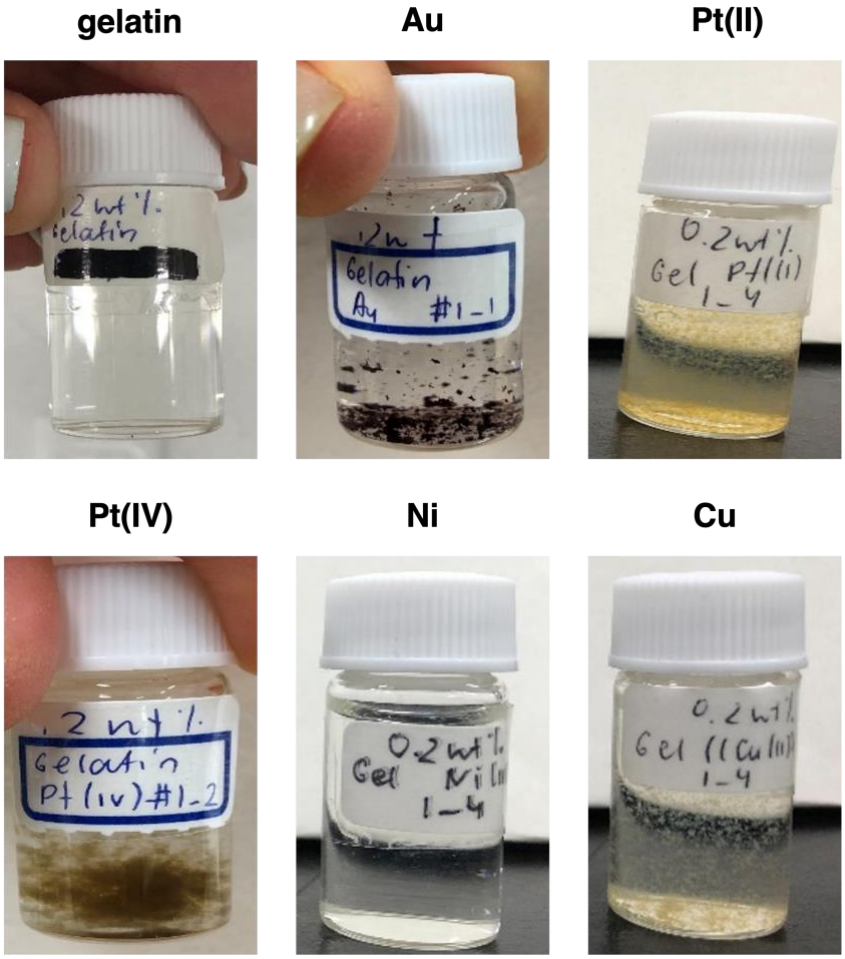

Figure S3. Pictures of metal/gelatin solutions after γ-ray irradiation

Stress-strain curve for Col-Pt(II)NP gel

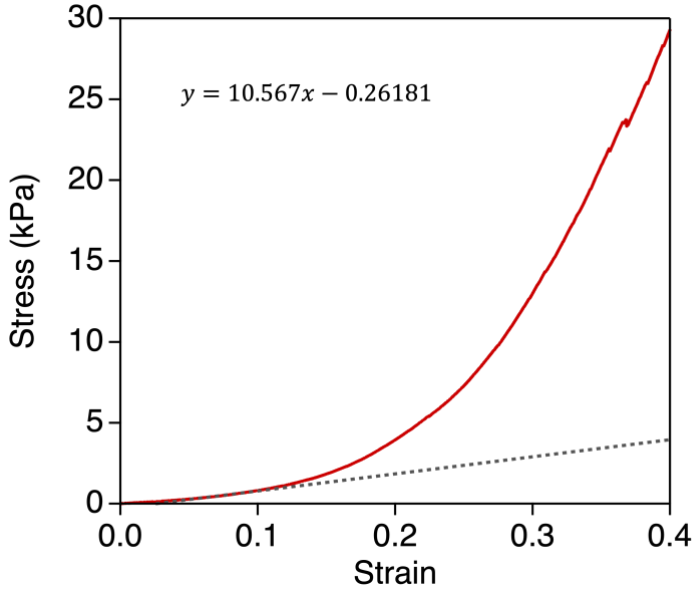

Figure S4. Stress–strain curve of Col-Pt(II)NP gel

***Note**: During the revision of this manuscript, the supply of the collagen sponge we originally used unfortunately stopped. Accordingly, the compression test for the stress-strain curve was performed using NMP collagen PS (Nippi Corp.) which exhibit similar transition-metal-induced gelation property.*

Laser set up of photothermal experiment

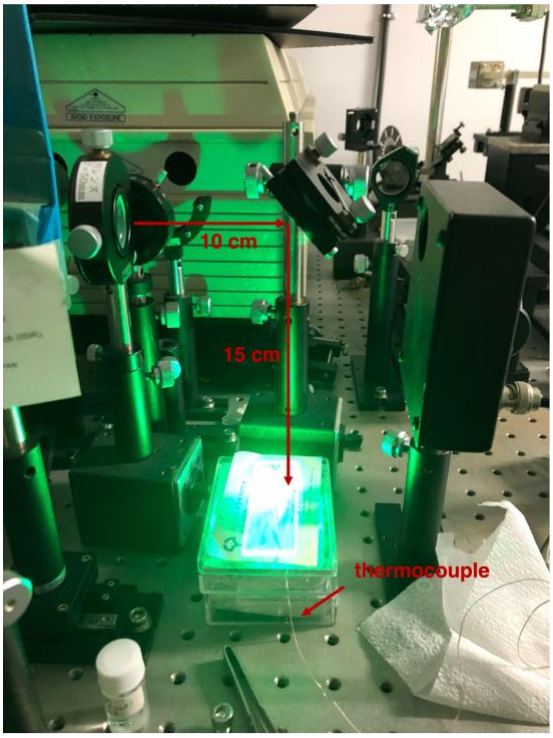

Figure S5. Laser setup for photothermal experiment