Collagen Recognizable Biocompatible Nanogelator for Tissue Engineering

Michiya Matsusaki,[1,2] Ryotaro Amekawa,[1] Tomonori Waku,[1] Yuji Tanaka,[3]
Akira Kubota,[3] Koji Nishida,[3] and Mitsuru Akashi[1]

[1]Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita 565-0871, Japan
[2]PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi 332-0012, Japan
[3]Department of Ophthalmology and Visual Science, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

Statement of Purpose: Recently, biocompatible and biodegradable injectable gelators, which can form stable hydrogels in living body, have attracted much attention in drug delivery system (DDS) and tissue engineering fields. Since driving force of the conventional injectable gelators is thermosensitivity,[1] stereocomplex formation,[2] and hydrogen bonding formation[3], it is difficult to present targeting property for specific tissues and diseases. Novel biocompatible gelators, which can form hydrogel at the presence of targeting biomolecules or tissues, will be useful for biomedical application.

We focused on collagen model peptide, proline-hydroxyproline-glycine (POG), for fabrication of collagen recognizable gelator. Yu et al. reported that gold nanoparticles with POG peptides can selectively adsorbed on collagen fibrils.[4] The collagen recognizable gelator is expected to be a powerful candidate for specific DDS or tissue engineering at diseased part containing collagen. In the present study, we synthesized $(POG)_{10}$ conjugated 4-arm-poly(ethylene glycol) (4-arm-PEG-$(POG)_{10}$) (Figure 1) and evaluated the gelation property of the 4-arm-PEG-$(POG)_{10}$ with collagen and other biomolecules.

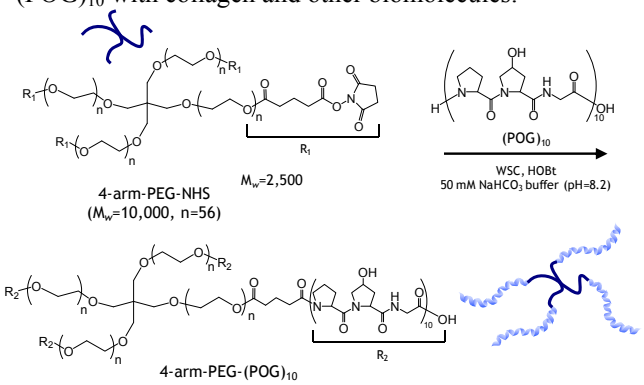

Figure 1. Synthetic scheme of 4-arm-PEG-$(POG)_{10}$.

Methods: Briefly, 50 mg (16.4 µmol) of $(POG)_{10}$ was dissolved in 50 mM sodium carbonate buffer (pH=8.2), and 41 mg (4.1 µmol) of 4-arm-PEG-NHS, 6.3 mg of WSC (32.8 µmol), and 4.4 mg (32.8 µmol) of HOBt were added to the buffer. After 24 hours of reaction at 25 °C, reaction solution was purified by dialysis for 48 hours and then final product was collected by freeze-dry for 3 days. The chemical structure of 4-arm-PEG-$(POG)_{10}$ was analyzed by ^{1}H-NMR, FT-IR, Mass spectra (yield 80%).

Results: The obtained 4-arm-PEG-$(POG)_{10}$ (PEG-POG) solution revealed positive peak at 225 nm by circular dichroism (CD) spectrum measurement, suggesting intermolecular triple helix formation. The triple helix was dissociated at 80 °C and the helix formation was reversible. Moreover, the PEG-POG formed reversible 10 nm of nano- constructs by triple helix formation. Interestingly, when the PEG-POG solution was mixed with type I collagen solution, the solution changed drastically to hydrogel within a few seconds. The storage modulus (G') of the collagen gel was 2370 Pa. Such gelation property of the PEG-POG was observed in only collagen solution (Figure 2a-i). Furthermore, gelation property of the PEG-POG was found in only fibrous collagens (type I, II, and III). Type IV and V collagens did not show any gelation. The detailed gelation mechanism is not clarified yet, but triple helix formation and helix-helix interaction might be important factors for the collagen recognizable gelation (Figure 2j).

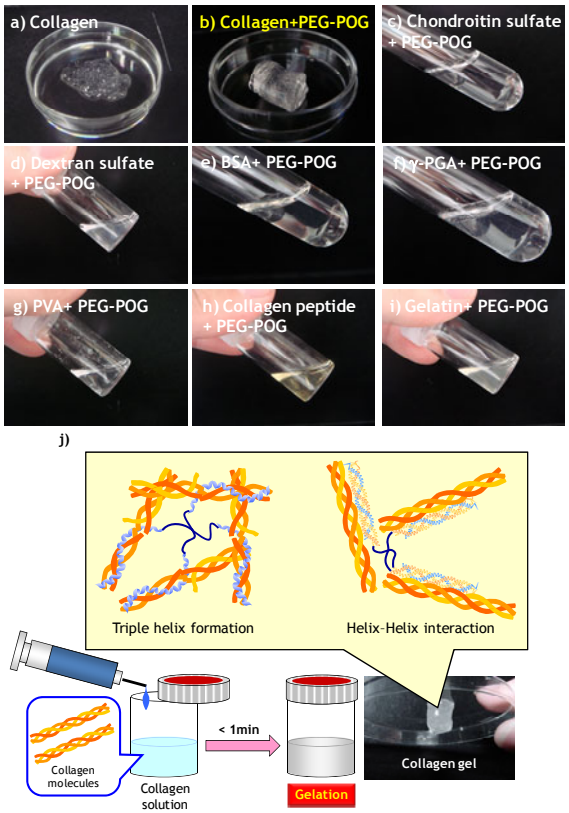

Figure 2. Photographs of various biomolecule solutions after mixing with the PEG-POG solution (a-i). Estimated gelation mechanism of collagen with PEG-POG (j).

Conclusions: We synthesized novel collagen recognizable biocompatible gelator based on collagen model peptide, $(POG)_{10}$. The PEG-POG would be one of the attractive nanomaterials as a fibrous collagen specific gelator for DDS and tissue engineering.

Acknowledgement. This work was supported by an Industrial Technology Research Grant Program in 2008 (08C46020a) from NEDO of Japan.

References: 1) Kim S. W. et al., Nature 1997;388:860. 2) Kimura Y. et al., Macromol. Biosci. 2001;1:204. 3) Ishihara K. et al., J. Biomed. Mater. Res. 2007;80A:45. 4) Yu S. M. et al., Angew. Chem. Int. Ed. 2006;45:2267.