In situ forming and nitric oxide releasing gelatin-based hydrogels for tissue engineering

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Statement of Purpose: *In situ* forming hydrogels have been widely used as bioactive materials for tissue regeneration, owing to their extracellular matrix mimicking properties and minimally invasive surgical procedure. Currently, nitric oxide (NO), an endogenous gas molecule, have been indicated as critical modulators for various therapeutic applications, such as treatment of vascular disorders, wound healing, and cancer treatment. However, the short half-life of NO in living systems are great challenges to its clinical applications. Herein, copper (Cu) ions (a NO-generating catalyst) were incorporated in the phenol-rich gelatin-based hydrogel (GH/Cu) for *in situ* generations of NO in the presence of endogenous S-nitrosothiol. The effect of NO releasing hydrogels on promoting cell functions (proliferation, migration, differentiation…) are thoroughly evaluated.

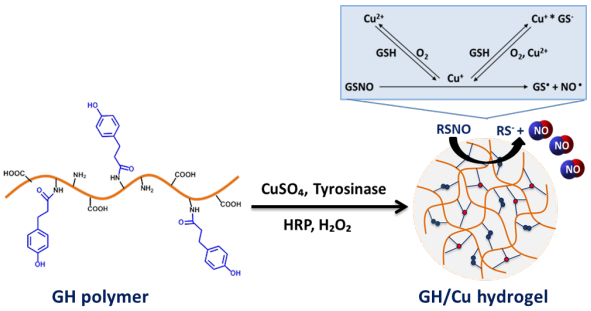

Figure 1. Schematic illustration for preparation of NO-releasing gelatin hydrogel

Methods: Phenol conjugated gelatin polymer (GH) was synthesized by coupling 3-(4-hydroxyphenyl) propionic acid onto gelatin backbone, using the EDC/NHS-mediated reaction. The NO-releasing hydrogels were synthesized by mixing GH solutions dissolved with enzyme (HRP and Tyr) with GH solutions dissolved with H_2O_2 and $CuSO_4$. Under catalyzation of tyrosinase and horseradish peroxidase, GH will be oxidized for the formation of a crosslinked network and the deposition of Cu nanoparticles (Figure 1). The storage moduli of hydrogels were measured using a rheometer. Modified Griess assay were used to analyze the release kinetics of NO from hydrogels, in the presence of NO donor. The effect of released NO on cell viability and tube formation was carried out using human umbilical vein endothelial cells (HUVECs). Besides, the *ex ovo* angiogenesis effect of hydrogel was investigated by Chick Chorioallantoic Membrane (CAM) assay. Furthermore, *in vivo* activity on vascular formation of NO-releasing hydrogels were also evaluated.

Results: The gelation time, mechanical properties and degradation of hydrogels was easily controlled by varying concentrations of HRP and H_2O_2. *In vitro* release studies indicated that the release behavior of NO from the hydrogel matrices can be precisely controlled in a wide range, for over 2 weeks. by varying the concentrations of $CuSO_4$. And the small release amount of Cu ions did not cause cytotoxic effect on HUVECs. Interestingly, the optimal NO release concentrations from hydrogels could stimulate the migration and tube formation activities of HUVECs. The results from *ex ovo* CAM assay, *in vivo* subcutaneous injection indicated that NO releasing hydrogels promoted the neovascularization and host tissue infiltration via material–tissue interactions.

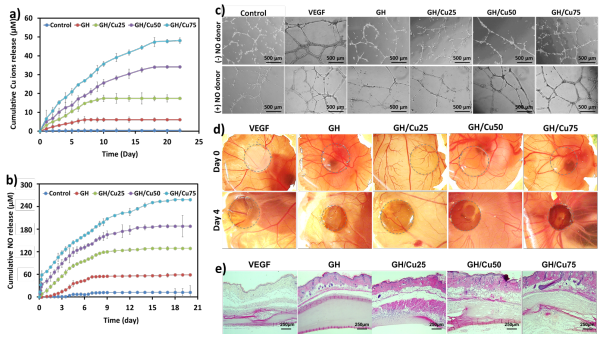

Figure 2. Cumulative release of Cu ions (a) and NO (b). Effect of NO release from hydrogels on (c) *in vitro* tube formation, (d) *ex ovo* CAM assay and (e) *in vivo* subcutaneous injection in rat model

Conclusions: We are successful to develop NO-releasing gelatin-based hydrogels as injectable and dynamic matrices for tissue regenerative applications. The concentration of NO release was accurately controlled by simply varying the $CuSO_4$ concentration. By optimizing the NO release amount from hydrogels, we can modulate the cellular functions for various biomedical applications, including wound healing, vascular disorder, anti-infection and stem cell based therapeutic products.

References:

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[2] Le TP, et al. J Mater Chem B 2017, 5, 757-764

Acknowledgements: This work was supported by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: 202012A01)