Supporting Information

An injectable and physical levan-based hydrogel as a dermal filler for soft tissue augment

Won Il Choi[†a], Youngmin Hwang[†b], Abhishek Sahu[b], Kiyoon Min[b], Daekyung Sung[a], Giyoong Tae[*b], Jeong Ho Chang[*a]

[a] Center for Convergence Bioceramic Materials, Convergence R&D Division, Korea Institute of Ceramic Engineering and Technology, 202, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju, Chungbuk 28160, Republic of Korea.

[b] School of Materials Science and Engineering, Gwangju Institute of Science and Technology, 123 Cheomdan-gwagiro, Buk-gu, Gwangju 61005, Republic of Korea

* To whom the correspondence should be made

E-mail: gytae@gist.ac.kr / Phone: 82-62-715-2305 / Fax: 82-62-715-2304

E-mail: jhchang@kicet.re.kr / Phone: 82-43-913-1501 / Fax: 82-43-913-1597

[1] These authors contributed equally to this paper.

***Keywords*:** Levan; Hyaluronic Acid; Hydrogel; Dermal filler; Wrinkle

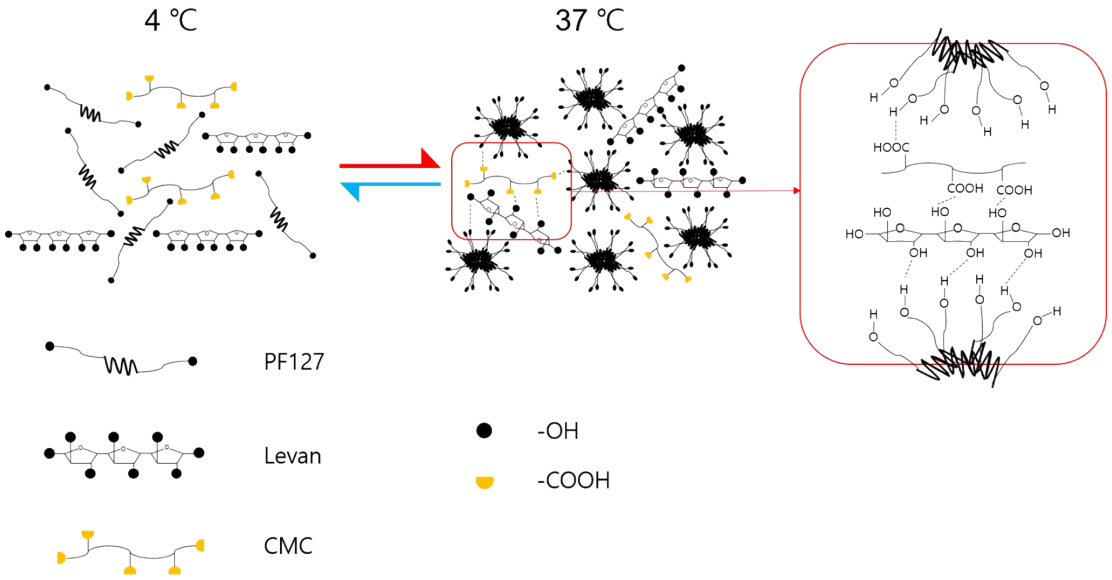

Figure S1. Scheme of the preparation of the levan-based hydrogel. Carboxyl groups of CMC and hydroxyl groups of levan and PF127 interacted with each other.

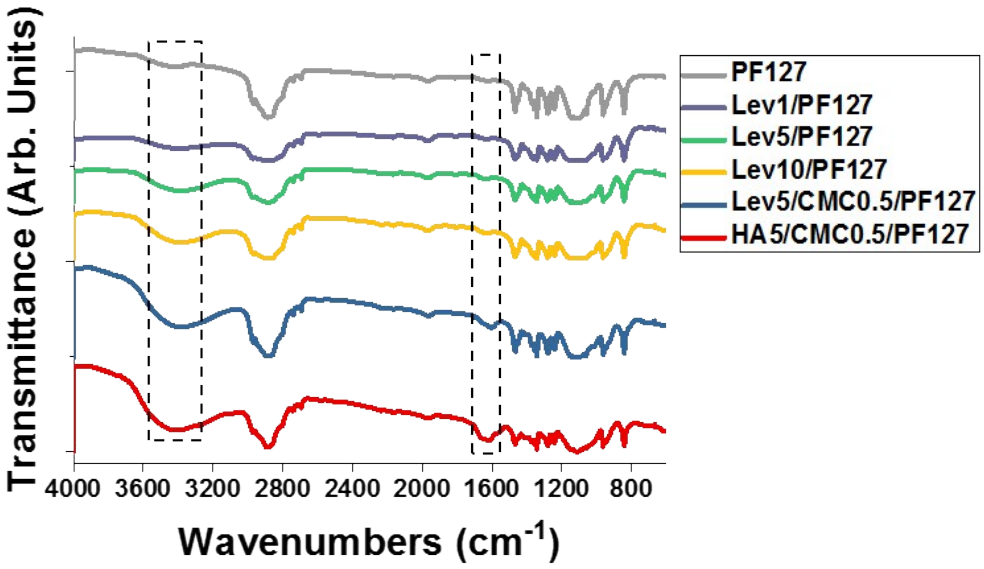

Figure S2. FT-IR spectra of levan hydrogels with different concentrations of levan and CMC (n = 3).

1) Cell cytotoxicity assay of levan

Before analyzing the cell cytotoxicity of levan hydrogels, the cell cytotoxicity of

levan itself was measured by CCK-8 assay.[1] Briefly, human adult dermal fibroblasts (hADF, 5 x 10^4 cells) were seeded on a 96 well tissue culture plate and incubated at 37 °C for 24 hours in the cell culture medium (DMEM containing 10 % FBS and 1 % penicillin/streptomycin). Then, various concentrations of levan and HA ranging from 1 to 5000 μg/mL was added to the cell and incubated at 37 °C for 24 hours. After incubation, the cell culture medium was changed to the fresh medium containing CCK-8 assay reagent and further incubated at 37 °C for 1 hour. The absorbance of the colored medium was measured at 450 nm by using a scanning multi-well spectrophotometer (SpectraMax, Molecular Devices, Sunnyvale, CA, USA). As a positive control group, poly(ethyleneimine) (PEI) was used on the cells in the same condition.

As expected, HA at all concentration (1 to 5000 μg/mL) exhibited no cytotoxicity in human dermal fibroblasts (Figure S3). As a new filling material, levan also showed no cytotoxicity even at a high concentration of 5 mg/mL, indicating that levan is biocompatible. Moreover, the value of the positive control group (PEI) was less than 5% which proves that the tests were performed correctly.

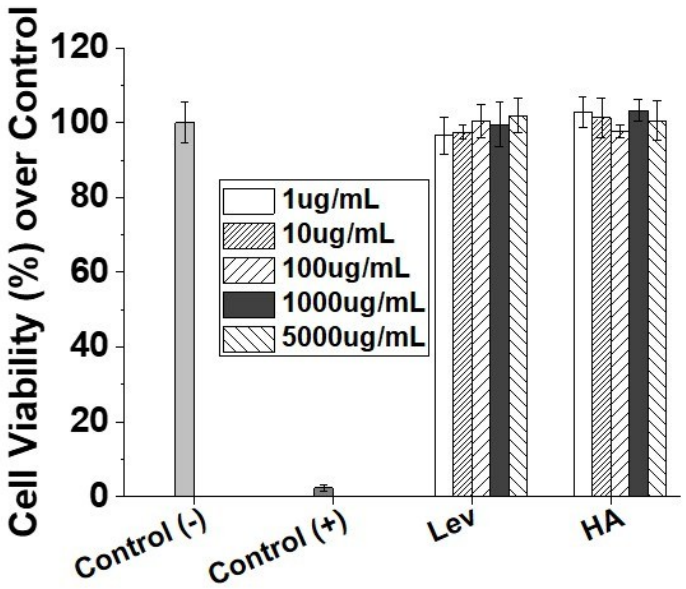

Figure S3. Cell cytotoxicity of levan against human adult dermal fibroblasts (hADF) compared to HA. The cell viability was measured by CCK-8 assay. Control (-): only cells, Control (+): cells treated with PEI as a toxic material (n = 3).

2) Effect of levan on cell proliferation and collagen production

Cell proliferation of hADF with levan was quantified by CCK-8 assay compared to HA. Different concentrations (0.2 and 0.5 wt%) of levan and HA were added to hADF and cultured 1, 3, and 4 days in cell culture medium (DMEM containing 10% FBS and 1% penicillin/streptomycin). At each time point, the cell culture medium was changed to fresh cell culture medium containing a CCK-8 reagent and incubated for 1 hour at 37 °C. After further 1 h incubation at 37 °C, the colorimetric absorbance of the produced formazan in the culture plate was measured at 450 nm (n = 3).

The collagen formation of hADF after treatment of levan was quantified by using

RT-PCR. After 1 day culture of hADF with levan (0.1 ~ 0.5 wt%) at 37 °C in the cell culture medium, cells were lysed by Trizol reagent and mRNA was isolated by PureLink RNA mini kit. Then, cDNA synthesis was conducted by using AccuPower RocketScript Cycle RT premix and finally, collagen type I gene (Col1A1) was quantified by using RT-PCR with iQ SYBR Green Supermix and the gene expression was calculated by the 2-(ΔΔCt) method. Expression value of Col1A1 was normalized to the beta-actin (β-actin, housekeeping gene) expression (n=4). The sequence of the β-actin and Col1A1 primers were as follows[2]: β-actin: 5'-ACTACCTTCAAGCCATC-3', 5'-TGATCTTGATCTTCATTGTG-3'; Col1A1: 5'-GGGATTCCCTGGACCTAAAG-3', 5'-GGAACACCTCGCTCTCCAG-3'.

As a result, the cell proliferation effect of levan was almost the same with HA which is well known as an effective dermal filling material for stimulating cell proliferation (Figure S4). Especially, the levan showed a remarkable cell proliferation effect in the dermal fibroblast cells since 3 days incubation. Interestingly, the levan resulted in the improved collagen production with increasing concentrations from 0.1 to 0.5 wt% and importantly the collagen formation effect of levan was higher than HA at 0.5 wt% (Figure S5). This suggests that levan has high potential as a material of effective dermal filler compared to HA, which is a commercialized filling material.

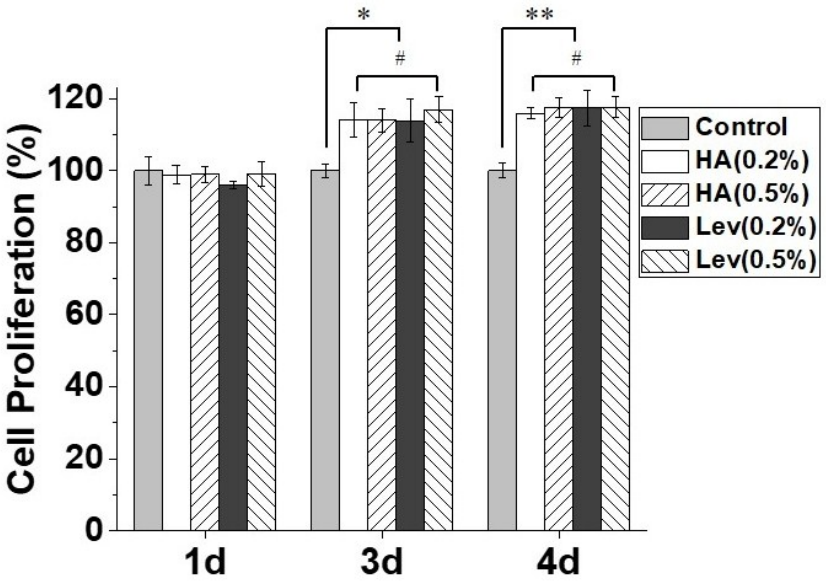

Figure S4. Cell proliferation effect of levan on human adult dermal fibroblasts (hADF) for 4 days compared to HA, analyzed by CCK-8 assay (mean±SEM, n = 3, * $p < 0.05$ and ** $p < 0.01$ in comparison between control and each group, and $^{\#}$ $p > 0.05$ in comparison between Lev and HA).

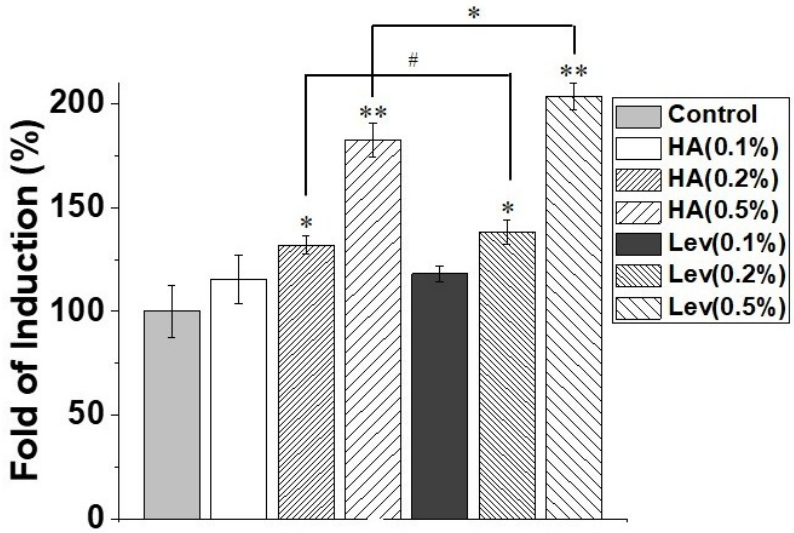

Figure S5. Quantitative collagen gene expression from human adult dermal fibroblasts (hADF) treated with levan and HA (mean±SEM, n = 4, * $p < 0.05$ and ** $p < 0.01$ in comparison between control and each group, and $^{\#}$ $p > 0.05$).

References

1. M. Kim, Y. Hwang and G. Tae, *Int. J. Biol. Macromol.* 2016, **93** 1603.
2. M.R. Monteiro, I.L.S. Tersario, S.V. Lucena, G.E.D.D. Moura and D. Steiner, *Surg. Cosmet. Dermatol.* 2013, **5** 222.