**Supplementary Materials**

*Article*

# Newly Designed Human-like Collagen to Maximize Sensitive Release of BMP-2 for Markedly Repairing of Bone Defect

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**Figure S1. Mercury porosimetry report of the HLC scaffold** The porosity of the HLC scaffold was over 99%

**Figure S2. Micro-CT images of the rat cranial defect repair** Micro-CT images of control, HLC and HLC-BMP implants groups

**Table S1.** Expression of the recombinant human-like collagens

**Table S2.** Original data of rhBMP-2 Release Kinetics.

**Table S3.** Original data of quantitative estimation of OPN and Runx-2 expression in each implant 2 weeks and 4 weeks after implantation.

**Table S4.** Original data of quantitative estimation of OPN and Runx-2 expression in each implant 8 weeks after implantation.

**Table S5.** Comparison the commercial collagen sponge and the recombinant human-like collagen sponge

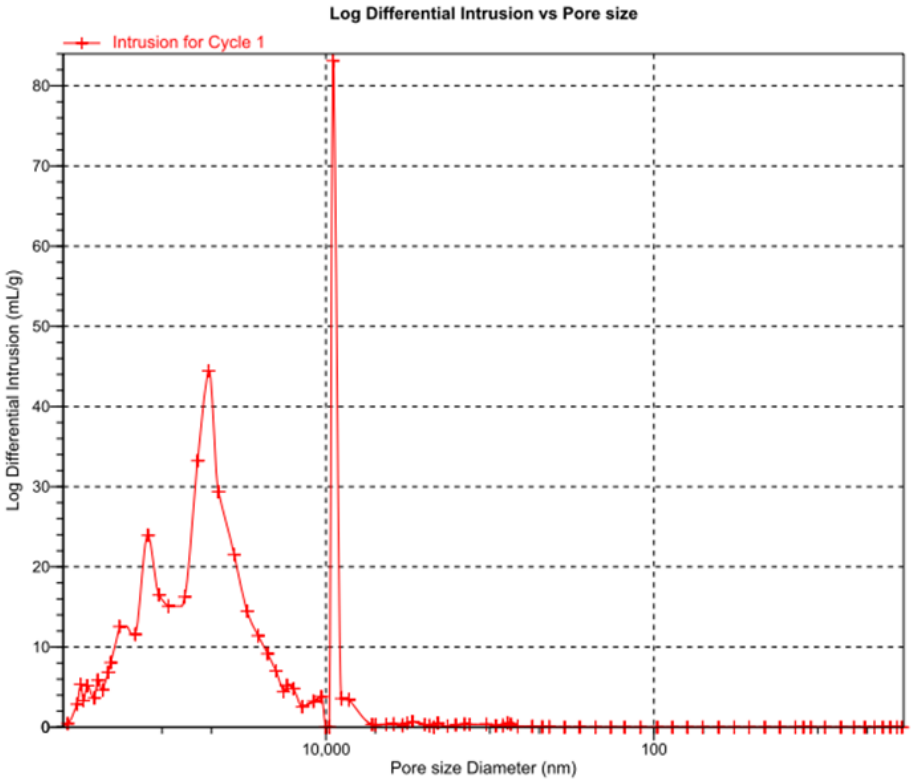
**MERCURY POROSIMETRY REPORT**

AutoPore IV 9500 V1.09 Serial: 239 Port: 1/1 Page 9

Sample ID: czy
Operator:
Submitter:
File: C:\9500\DATA\FL-CZY.SMP

LP Analysis Time: 1/5/2017 9:31:39AM
HP Analysis Time: 1/5/2017 10:15:54AM
Report Time: 1/5/2017 4:33:12PM

Sample Weight: 0.0292 g
Correction Type: None
Show Neg. Int: No




| Cumulative Pore Volume (mL/g) | % of Total Intrusion Volume |
|---|---|
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
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| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.1208 | 99.4510 |
| 24.0192 | 99.0320 |

**Figure S1. Mercury porosimetry report of HLC scaffold.** The porosity of the HLC scaffold was over 99%.

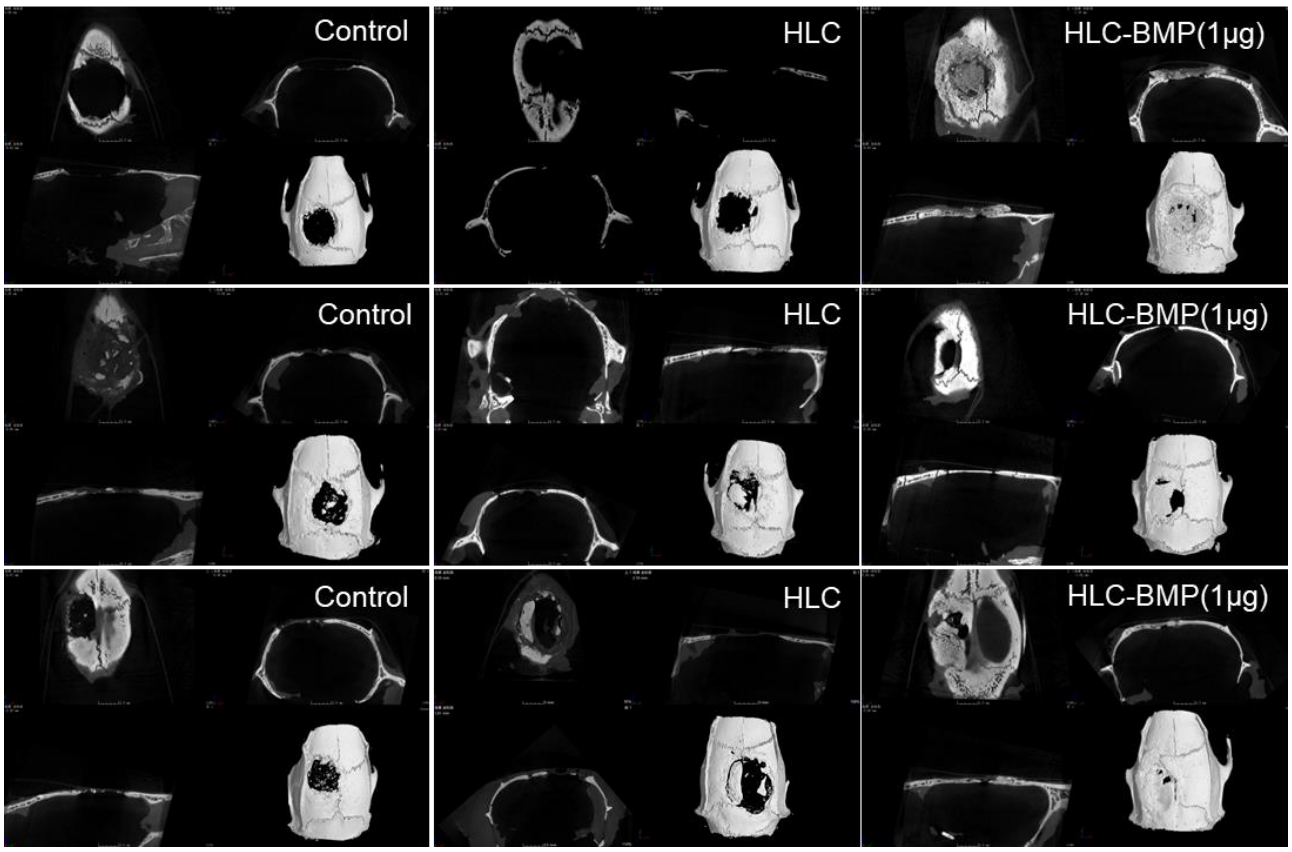


**Figure S2. Micro-CT images of the rat cranial defect repair** Micro-CT images of control, HLC and HLC-BMP implants groups

**Table S1. Expression of the recombinant human-like collagens**

| Host | Yield | Molecular Wt. | Ref. |
|---|---|---|---|
| *E. coli* origami (DE3) | 260 mg/L in a 10 L fermentor | 35 kDa | Tang *et al.* [1] |
| *E. coli* Rosetta (DE3) | Shake flask culture | 40 kDa | Yang *et al.*[2] |
| *E. coli* BL21 (DE3) | 14 g/L in a 500 L fermentor | 93 kDa | Fan *et al.* [3, 4] |

(1). Tang, Y.; Yang, X.; Hang, B.; Li, J.; Huang, L.; Huang, F.; Xu, Z. Efficient Production of Hydroxylated Human-Like Collagen Via the Co-Expression of Three Key Genes in Escherichia coli Origami (DE3). Applied Biochemistry & Biotechnology 2016, 178, 1458-1470.

(2). Yang, J.; Jie-Ying, Y. U.; Wang, M.; Shao-Min, W. U.; Yi, M. A.; Wang, J. F. Expression, Purification, and Functional Characterization of Recombinant Human-Like Type i Collagen Peptide in E.coli. Modern Food Science & Technology 2016.

(3). Fan, D. D.; Luo, Y.; Mi, Y.; Ma, X. X.; Shang, L. Characteristics of Fed-Batch Cultures of Recombinant Escherichia Coli Containing Human-Like Collagen cDNA at Different Specific Growth Rates. Biotechnology Letters 2005, 27, 865-870.

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**Table S2. Original data of rhBMP-2 Release Kinetics.** Day 1~3: Burst release period; Day 3~28 and later: Sustained release period. The percentage of rhBMP-2 release is no difference among loading with different dose rhBMP-2 groups at each time point ($p > 0.05$, n=4).

| **Time (Days)** | **HLC-BMP 1μg (%)** | | | | **Average** | **STD** |
|---|---|---|---|---|---|---|
| 1 | 11.75 | 9.47 | 7.96 | 9.78 | 9.74 | 1.56 |
| 2 | 13.60 | 8.66 | 14.75 | 14.47 | 12.87 | 2.85 |
| 3 | 16.80 | 19.57 | 15.58 | 20.59 | 18.13 | 2.34 |
| 5 | 44.77 | 26.78 | 34.68 | 29.88 | 34.03 | 7.86 |
| 7 | 35.76 | 36.80 | 37.60 | 42.69 | 38.21 | 3.08 |
| 14 | 43.57 | 40.70 | 40.68 | 46.59 | 42.88 | 2.82 |
| 21 | 32.85 | 44.46 | 56.54 | 41.73 | 43.89 | 9.78 |
| 28 | 40.79 | 45.69 | 58.69 | 33.99 | 44.79 | 10.43 |
| **Time (Days)** | **HLC-BMP 2μg (%)** | | | | **Average** | **STD** |
| 1 | 15.65 | 8.57 | 14.48 | 9.64 | 12.09 | 3.50 |
| 2 | 19.69 | 15.77 | 19.58 | 16.76 | 17.95 | 1.99 |
| 3 | 17.54 | 15.58 | 23.77 | 25.80 | 20.67 | 4.89 |
| 5 | 46.67 | 29.68 | 31.70 | 39.81 | 36.96 | 7.81 |
| 7 | 51.57 | 39.55 | 40.76 | 32.68 | 41.14 | 7.81 |
| 14 | 41.80 | 46.64 | 42.55 | 50.57 | 45.39 | 4.05 |
| 21 | 45.66 | 50.46 | 42.67 | 44.65 | 45.86 | 3.31 |
| 28 | 50.43 | 43.90 | 49.12 | 40.32 | 45.94 | 4.69 |
| **Time (Days)** | **HLC-BMP 3μg (%)** | | | | **Average** | **STD** |
| 1 | 9.56 | 8.65 | 13.77 | 15.86 | 11.96 | 3.43 |
| 2 | 17.85 | 15.80 | 10.57 | 9.68 | 13.47 | 3.97 |
| 3 | 23.90 | 22.75 | 21.85 | 17.70 | 21.55 | 2.70 |
| 5 | 34.55 | 36.80 | 35.46 | 40.70 | 36.88 | 2.71 |
| 7 | 45.60 | 38.59 | 34.79 | 39.90 | 39.72 | 4.48 |
| 14 | 45.89 | 36.78 | 49.59 | 42.77 | 43.76 | 5.42 |
| 21 | 45.85 | 40.78 | 54.86 | 42.09 | 45.90 | 6.35 |
| 28 | 43.87 | 50.45 | 44.86 | 47.98 | 46.79 | 3.00 |
| **Time (Days)** | **HLC-BMP 4μg (%)** | | | | **Average** | **STD** |
| 1 | 16.58 | 18.59 | 9.48 | 15.47 | 15.03 | 3.92 |
| 2 | 16.69 | 17.69 | 22.46 | 17.58 | 18.61 | 2.61 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 27.54 | 18.40 | 26.50 | 19.70 | 23.04 | 4.65 |
| 5 | 39.35 | 36.65 | 39.33 | 38.54 | 38.47 | 1.27 |
| 7 | 42.37 | 38.16 | 38.50 | 39.59 | 39.66 | 1.91 |
| 14 | 45.48 | 49.84 | 48.27 | 48.37 | 47.99 | 1.82 |
| 21 | 47.80 | 47.80 | 49.77 | 47.68 | 48.26 | 1.01 |
| 28 | 51.69 | 49.78 | 50.59 | 39.70 | 47.94 | 5.55 |
| **Time (Days)** | **HLC-BMP 5µg (%)** | | | | **Average** | **STD** |
| 1 | 16.58 | 18.59 | 9.48 | 15.47 | 15.03 | 3.92 |
| 2 | 16.69 | 17.69 | 22.46 | 17.58 | 18.61 | 2.61 |
| 3 | 27.54 | 18.40 | 26.50 | 19.70 | 23.04 | 4.65 |
| 5 | 39.35 | 36.65 | 39.33 | 38.54 | 38.47 | 1.27 |
| 7 | 42.37 | 38.16 | 38.50 | 39.59 | 39.66 | 1.91 |
| 14 | 45.48 | 49.84 | 48.27 | 48.37 | 47.99 | 1.82 |
| 21 | 45.80 | 55.80 | 49.77 | 43.68 | 48.76 | 5.33 |
| 28 | 59.69 | 39.78 | 43.59 | 49.70 | 48.19 | 8.69 |

**Table S3. Original data of quantitative estimation of OPN and Runx-2 expression in each implant 2 weeks and 4 weeks after implantation.**

| Time (weeks)/group | | OPN (%) | | | Average | STD |
|---|---|---|---|---|---|---|
| 2 | Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC-BMP | 21.07 | 14.13 | 18.77 | 17.99 | 3.54 |
| 4 | Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC | 0.26 | 0.18 | 0.24 | 0.23 | 0.04 |
| | HLC-BMP | 24.77 | 16.61 | 22.07 | 21.15 | 4.16 |
| **Time (weeks)/group** | | **Runx-2 (%)** | | | **Average** | **STD** |
| 2 | Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC-BMP | 4.97 | 3.33 | 4.43 | 4.25 | 0.83 |
| 4 | Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | HLC | 0.31 | 0.21 | 0.27 | 0.26 | 0.05 |
| | HLC-BMP | 8.60 | 5.77 | 7.67 | 7.35 | 1.44 |

**Table S4. Original data of quantitative estimation of OPN and Runx-2 expression in each implant 8 weeks after implantation.**

| **Group** | **OPN (%)** | | | **Average** | **STD** |
|---|---|---|---|---|---|
| Control | 0.09 | 0.06 | 0.08 | 0.08 | 0.02 |
| HLC- 1μg BMP-2 dimer | 35.60 | 23.87 | 31.72 | 30.39 | 5.98 |
| HLC- 5μg BMP-2 dimer | 43.98 | 29.48 | 39.18 | 37.55 | 7.38 |
| **Group** | **Runx-2 (%)** | | | **Average** | **STD** |
| Control | 0.43 | 0.29 | 0.38 | 0.36 | 0.07 |
| HLC- 1μg BMP-2 dimer | 18.66 | 12.51 | 16.62 | 15.93 | 3.13 |
| HLC- 5μg BMP-2 dimer | 23.75 | 15.92 | 21.16 | 20.27 | 3.99 |

**Table S5. Comparison the commercial collagen sponge and the recombinant human-like collagen sponge**

| Source | Company | Release rate | Effect |
|---|---|---|---|
| bovine achilles tendon -extracted collagen[5] | Helistat®, Integra Life Sciences Corporation , USA | 5 μg BMP-2 dose group released 55.46 ± 2.01% within 1 hour, 84.94 ± 8.68% within 1 day, and 100% at day 2. The 1 μg BMP-2 group released 34.73 ± 8.25% within 6 hours, and 45% at the third day. | The collagen sponge containing 1 μg BMP-2 formed the new bone volume was 0.6 ± 0.3 $mm^3$ at 8 weeks post-implantation into the rat muscle bag. |
| recombinant from *E. coli* | Our lab. | HLC containing 5 μg BMP-2 gel released 15.03 ± 3.92% of BMP-2 at day 1, 18.61 ± 2.61% at day 2 and 48.19 ± 8.69% at day 28. The HLC containing 1 μg BMP-2 released 9.74 ± 1.56% at day 1, 18.13 ± 2.34% at day 3, and 44.79 ± 10.43% at day 28. | Using the similar rat cranial defect repair model, the repair rate is 65.21 ± 4.44% at 2 weeks postoperatively. At 4 weeks postoperatively, cranial defect repair rate of the designed HLC loaded with 1 μg BMP-2 group achieved 79.95 ± 7.57%. |
| bovine skin -extracted collagen[6] | I-PC, Koken Co. Ltd. Tokyo, Japan | Collagen gel containing 60 μg of BMP-2 released 6% of BMP-2 at day 1. About 33% of the total BMP-2 was released from the collagen gel within 15 days. | None of the new bone tissue appeared in the mouse cranial defect area at 2 weeks postoperatively. At 4 weeks postoperatively, the cranial defect repair rate of the commercial sponge loaded with almost 1 μg BMP-2 group was 40.24 ± 9.70%. |

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