

# Osteogenic potential of human-derived advanced platelet-rich fibrin and albumin platelet-rich fibrin: An *in vitro* study

## Supplementary File

**Table S1.** Primer sequences used for RT-qPCR

| No. | Gene         | Forward primer      | Reverse primer        |
|-----|--------------|---------------------|-----------------------|
| 1   | <i>GAPDH</i> | GAAGGTGAAGGTCGGAGTC | GAAGATGGTGATGGGATTTTC |
| 2   | <i>BGLAP</i> | AGGTGCAGCCTTGTGTCCA | TGTGGTCAGCCAACTCGTCA  |

Abbreviation: RT-qPCR: Real-time quantitative polymerase chain reaction.

**Table S2.** MTT assay results for MG-63 cell proliferation (%) after treatment with DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium at 7, 14, and 21 days

| Days   | Control                             |         |         |                                       |         |         |
|--------|-------------------------------------|---------|---------|---------------------------------------|---------|---------|
|        | Value 1                             | Value 2 | Value 3 |                                       |         |         |
| Day 7  | 62.71                               | 63.29   | 64.45   |                                       |         |         |
| Day 14 | 70.66                               | 70.80   | 71.24   |                                       |         |         |
| Day 21 | 79.91                               | 80.34   | 81.35   |                                       |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
|        | A-PRF-conditioned medium (Sample 1) |         |         | Alb-PRF-conditioned medium (Sample 1) |         |         |
| Day 7  | 86.74                               | 84.58   | 85.23   | 97.10                                 | 96.76   | 96.19   |
| Day 14 | 115.50                              | 114.94  | 113.62  | 118.47                                | 118.29  | 119.66  |
| Day 21 | 94.16                               | 92.67   | 94.32   | 119.01                                | 118.99  | 118.04  |
|        | A-PRF-conditioned medium (Sample 2) |         |         | Alb-PRF-conditioned medium (Sample 2) |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
| Day 7  | 89.87                               | 88.18   | 88.63   | 111.15                                | 111.33  | 109.73  |
| Day 14 | 114.73                              | 115.34  | 114.66  | 120.41                                | 121.13  | 120.82  |
| Day 21 | 91.25                               | 91.31   | 91.92   | 124.43                                | 123.64  | 123.68  |
|        | A-PRF-conditioned medium (Sample 3) |         |         | Alb-PRF-conditioned medium (Sample 3) |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
| Day 7  | 78.11                               | 78.36   | 78.49   | 90.09                                 | 90.41   | 89.23   |
| Day 14 | 104.70                              | 104.59  | 104.01  | 111.84                                | 112.13  | 112.42  |
| Day 21 | 90.09                               | 90.41   | 89.23   | 114.73                                | 114.88  | 115.31  |

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

**Table S3.** Alkaline phosphatase activity in MG-63 cells (IU/L) cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium at 7, 14, and 21 days

| Days   | Control                             |         |         |                                       |         |         |
|--------|-------------------------------------|---------|---------|---------------------------------------|---------|---------|
|        | Value 1                             | Value 2 | Value 3 |                                       |         |         |
| Day 7  | 3.26                                | 3.23    | 3.21    |                                       |         |         |
| Day 14 | 4.94                                | 4.96    | 4.96    |                                       |         |         |
| Day 21 | 4.31                                | 4.36    | 4.38    |                                       |         |         |
|        | A-PRF-conditioned medium (Sample 1) |         |         | Alb-PRF-conditioned medium (Sample 1) |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
| Day 7  | 3.35                                | 3.46    | 3.33    | 3.46                                  | 3.47    | 3.49    |
| Day 14 | 6.04                                | 5.96    | 6.10    | 6.46                                  | 6.41    | 6.44    |
| Day 21 | 5.26                                | 5.19    | 5.23    | 6.36                                  | 6.07    | 6.38    |
|        | A-PRF-conditioned medium (Sample 2) |         |         | Alb-PRF-conditioned medium (Sample 2) |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
| Day 7  | 3.44                                | 3.28    | 3.48    | 3.70                                  | 3.63    | 3.64    |
| Day 14 | 5.07                                | 5.13    | 5.03    | 6.83                                  | 6.81    | 6.79    |
| Day 21 | 4.68                                | 4.61    | 4.63    | 6.35                                  | 6.33    | 6.30    |
|        | A-PRF-conditioned medium (Sample 3) |         |         | Alb-PRF-conditioned medium (Sample 3) |         |         |
|        | Value 1                             | Value 2 | Value 3 | Value 1                               | Value 2 | Value 3 |
| Day 7  | 2.48                                | 2.49    | 2.50    | 2.6                                   | 2.6     | 2.68    |
| Day 14 | 5.92                                | 6.09    | 6.27    | 6.27                                  | 6.33    | 6.54    |
| Day 21 | 4.42                                | 4.56    | 4.71    | 6.18                                  | 6.20    | 6.13    |

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium.

## Osteogenic potential of PRF derivatives

**Table S4.** Ct values for *BGLAP* gene expression in MG-63 cells cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium on day 21

| No. | Ct value                            |                                       |
|-----|-------------------------------------|---------------------------------------|
|     | Control                             |                                       |
| 1   | 18.15                               |                                       |
| 2   | 18.28                               |                                       |
| 3   | 18.16                               |                                       |
|     | A-PRF-conditioned medium (Sample 1) | Alb-PRF-conditioned medium (Sample 1) |
| 1   | 13.78                               | 12.30                                 |
| 2   | 14.03                               | 12.61                                 |
| 3   | 14.14                               | 12.75                                 |
|     | A-PRF-conditioned medium (Sample 2) | Alb-PRF-conditioned medium (Sample 2) |
| 1   | 16.88                               | 13.74                                 |
| 2   | 16.79                               | 14.04                                 |
| 3   | 16.88                               | 13.69                                 |
|     | A-PRF-conditioned medium (Sample 3) | Alb-PRF-conditioned medium (Sample 3) |
| 1   | 14.61                               | 13.87                                 |
| 2   | 13.86                               | 13.15                                 |
| 3   | 14.33                               | 13.75                                 |

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium.

**Table S5.** Intergroup comparison of mean BGLAP expression in MG-63 cells cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium on day 21, as assessed by one-way ANOVA

| Group                      | n | Mean  | Standard deviation | ANOVA p-value |
|----------------------------|---|-------|--------------------|---------------|
| Control                    | 3 | 1.088 | 0.081              | <0.001*       |
| A-PRF-conditioned medium   | 3 | 1.489 | 0.081              |               |
| Alb-PRF-conditioned medium | 3 | 2.309 | 0.071              |               |
| Total                      | 9 | 1.629 | 0.543              |               |

Note: \*The mean difference is significant at the 0.05 level.

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium; ANOVA: Analysis of variance.

**Table S6.** Mineralized nodule formation in MG-63 cells cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium on day 21

| Group                                 | Concentration (μM/mL) |         |         | Mean value  |
|---------------------------------------|-----------------------|---------|---------|-------------|
|                                       | Value 1               | Value 2 | Value 3 |             |
| Control                               | 176.3                 | 181.3   | 178.3   | 178.63±2.51 |
| A-PRF-conditioned medium (Sample 1)   | 402.3                 | 408.3   | 404.3   | 404.96±3.05 |
| A-PRF-conditioned medium (Sample 2)   | 408.3                 | 416.3   | 413.3   | 412.63±4.04 |
| A-PRF-conditioned medium (Sample 3)   | 622.3                 | 633.3   | 625.3   | 626.96±5.68 |
| Alb-PRF-conditioned medium (Sample 1) | 792.3                 | 806.3   | 803.3   | 800.63±7.37 |
| Alb-PRF-conditioned medium (Sample 2) | 813.3                 | 809.3   | 812.3   | 811.63±2.08 |
| Alb-PRF-conditioned medium (Sample 3) | 822.3                 | 833.3   | 829.3   | 828.3±5.56  |

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium.

**Table S7.** Intergroup comparison of mean mineralized nodule formation in MG-63 cells cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium on day 21, as assessed by one-way ANOVA

| Group                      | n | Mean   | Standard deviation | ANOVA p-value |
|----------------------------|---|--------|--------------------|---------------|
| Control                    | 3 | 178.63 | 2.51               | <0.001*       |
| A-PRF-conditioned medium   | 3 | 481.52 | 4.19               |               |
| Alb-PRF-conditioned medium | 3 | 813.52 | 3.71               |               |
| Total                      | 9 | 491.22 | 275.02             |               |

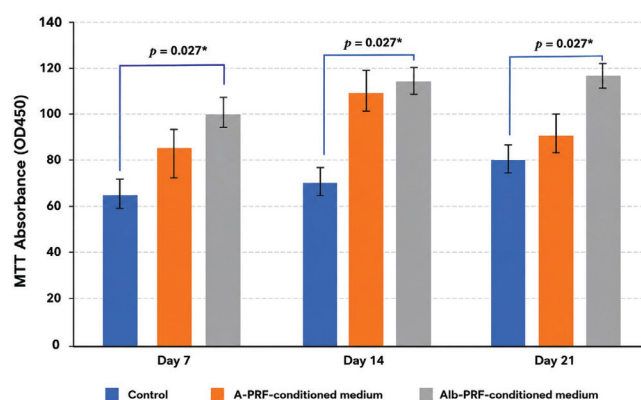
Note: \*The mean difference is significant at the 0.05 level.

Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium; ANOVA: Analysis of variance.

**Table S8.** PDGF-AA release (pg/mL) from A-PRF and Alb-PRF samples incubated in DMEM compared with DMEM alone (control) over 21 days

| Days   | Value 1                             | Value 2 | Value 1                               | Value 2  |
|--------|-------------------------------------|---------|---------------------------------------|----------|
|        | A-PRF-conditioned medium (Sample 1) |         | Alb-PRF-conditioned medium (Sample 1) |          |
| Day 1  | 16.43                               | 16.46   | 20.05                                 | 20.12    |
| Day 3  | 18.73                               | 18.31   | 21.58                                 | 21.22    |
| Day 7  | 20.28                               | 20.34   | 22.99                                 | 24.14    |
| Day 14 | 23.56                               | 24.48   | 25.46                                 | 26.73    |
| Day 21 | 15.30                               | 15.06   | 27.21                                 | 31.63    |
|        | A-PRF-conditioned medium (Sample 2) |         | Alb-PRF-conditioned medium (Sample 2) |          |
| Day 1  | 16.41                               | 16.06   | 15.93                                 | 17.63619 |
| Day 3  | 18.89                               | 17.08   | 20.21                                 | 22.15759 |
| Day 7  | 20.34                               | 21.69   | 23.85                                 | 16.0856  |
| Day 14 | 23.96                               | 25.07   | 26.95                                 | 26.13619 |
| Day 21 | 19.82                               | 21.64   | 28.56                                 | 28.04475 |
|        | A-PRF-conditioned medium (Sample 3) |         | Alb-PRF-conditioned medium (Sample 3) |          |
| Day 1  | 15.93                               | 17.63   | 22.05                                 | 22.12    |
| Day 3  | 17.89                               | 18.08   | 24.58                                 | 24.22    |
| Day 7  | 21.34                               | 23.69   | 26.99                                 | 27.14    |
| Day 14 | 24.96                               | 26.07   | 29.46                                 | 29.73    |
| Day 21 | 18.82                               | 19.64   | 31.21                                 | 33.63    |
|        | Value 1 (control)                   |         | Value 2 (control)                     |          |
| Day 1  | 8.33                                |         | 8.36                                  |          |
| Day 3  | 7.06                                |         | 7.13                                  |          |
| Day 7  | 9.31                                |         | 8.06                                  |          |
| Day 14 | 9.83                                |         | 10.53                                 |          |
| Day 21 | 8.43                                |         | 8.46                                  |          |

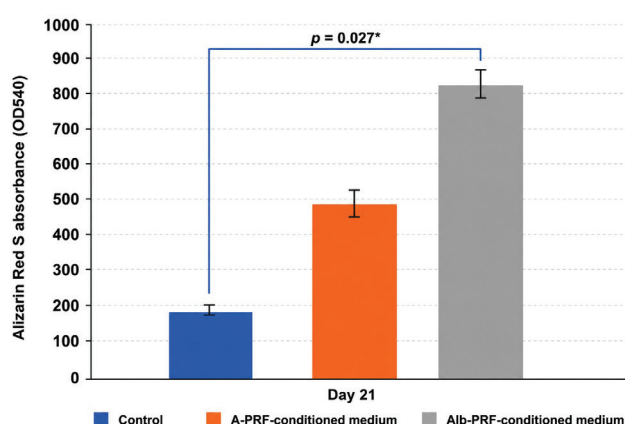
Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium.

**Figure S1.** MG-63 cell proliferation after culture in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium at 7, 14, and 21 days. Data are presented as mean  $\pm$  SD from three independent repeats. Statistical significance compared with the control group was analyzed using the Kruskal–Wallis test. \* $p < 0.05$ . Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium; SD: Standard deviation.**Table S9.** Intergroup comparison of mean PDGF-AA release levels (pg/mL) from A-PRF and Alb-PRF samples incubated in DMEM compared with DMEM alone (control) at 1, 3, 7, 14, and 21 days, as assessed by one-way ANOVA

| Group                      | <i>n</i> | Mean  | Standard deviation | ANOVA <i>p</i> -value |
|----------------------------|----------|-------|--------------------|-----------------------|
| Day 7                      |          |       |                    |                       |
| Control                    | 2        | 8.34  | 0.01               | <0.001*               |
| A-PRF-conditioned medium   | 2        | 16.49 | 0.32               |                       |
| Alb-PRF-conditioned medium | 2        | 19.65 | 0.43               |                       |
| Total                      | 6        | 14.83 | 5.22               |                       |
| Day 3                      |          |       |                    |                       |
| Control                    | 2        | 7.09  | 0.04               | <0.001*               |
| A-PRF-conditioned medium   | 2        | 18.16 | 0.48               |                       |
| Alb-PRF-conditioned medium | 2        | 22.33 | 0.28               |                       |
| Total                      | 6        | 15.86 | 7.04               |                       |
| Day 7                      |          |       |                    |                       |
| Control                    | 2        | 8.68  | 0.87               | <0.001*               |
| A-PRF-conditioned medium   | 2        | 21.28 | 0.88               |                       |
| Alb-PRF-conditioned medium | 2        | 23.53 | 1.52               |                       |
| Total                      | 6        | 17.83 | 7.21               |                       |
| Day 14                     |          |       |                    |                       |
| Control                    | 2        | 10.18 | 0.49               | <0.001*               |
| A-PRF-conditioned medium   | 2        | 24.68 | 0.74               |                       |
| Alb-PRF-conditioned medium | 2        | 27.41 | 0.17               |                       |
| Total                      | 6        | 20.76 | 8.29               |                       |
| Day 21                     |          |       |                    |                       |
| Control                    | 2        | 8.44  | 0.01               | <0.001*               |
| A-PRF-conditioned medium   | 2        | 18.38 | 0.56               |                       |
| Alb-PRF-conditioned medium | 2        | 30.05 | 1.48               |                       |
| Total                      | 6        | 18.96 | 9.69               |                       |

Note: \*The mean difference is significant at the 0.05 level.

PDGF: Platelet-derived growth factor; A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium.

**Figure S2.** Mineralized nodule formation in MG-63 cells cultured in DMEM alone (control), A-PRF-conditioned medium, or Alb-PRF-conditioned medium on day 21. Data are presented as mean  $\pm$  SD from three independent repeats. Statistical significance compared with the control group was analyzed using the Kruskal–Wallis test. \* $p < 0.05$ . Abbreviations: A-PRF: Advanced platelet-rich fibrin, Alb-PRF: Albumin platelet-rich fibrin; DMEM: Dulbecco's modified Eagle medium; SD: Standard deviation.