

## Supplemental Results: Gene Expression in the Soft Tissue Envelope Surrounding Bioreactor-Generated Tissues

The soft tissue envelope surrounding bioreactor-generated tissues was sampled to evaluate the expression of genes associated with markers of osteogenesis (runt related transcription factor 2 (*Runx2*) and *Osteocalcin*) and osteogenic growth factors (bone morphogenetic protein 2 (*BMP2*) and bone morphogenetic protein 7 (*BMP7*)), markers of chondrogenesis (SRY-box 9 (*Sox9*) and *Collagen II*), and angiogenic growth factors (basic fibroblast growth factor (*bFGF*) and vascular endothelial growth factor (*VEGF*)) (Supplemental Table S6).

Tissue from the envelopes was taken from the side adjacent to the periosteum, at the bottom of the bioreactor (“-B”), and from the side furthest from the periosteum, at the top of the bioreactor (“-T”). As positive and negative controls, samples from sheep skin and undisrupted periosteum, the source of mesenchymal stem cells that migrate into the bioreactors and produce viable bone tissue, were also analyzed. AG-T n = 6, AG-B n = 6, SG-T n = 8, SG-B n = 8, Skin n = 18, and Periosteum n = 18 for all genes, except *Runx2* for which Periosteum n = 16. Expression data as fold difference relative to *GAPDH* can be seen in Supplemental Figure 6. As a trend, AG groups (particularly AG-B) and Periosteum had greater expression of osteogenic, chondrogenic, and angiogenic genes; this was only statistically significant for AG-B and *Osteocalcin* (greater expression than all other groups,  $p < 0.05$ ).

**Supplemental Table S1.** Bioreactor-generated tissue radiographic analysis.

| <b>Assay</b>        | <b>Autograft</b> |               | <b>Synthetic Graft</b> |               |
|---------------------|------------------|---------------|------------------------|---------------|
| <b>Timepoint</b>    | <b>Week 0</b>    | <b>Week 9</b> | <b>Week 0</b>          | <b>Week 9</b> |
| <b>BV/TV (%)</b>    | 7.05 ± 4.30      | 42.29 ± 8.15  | 36.56 ± 1.83           | 43.72 ± 10.90 |
| <b>Tb.Th (mm)</b>   | 0.12 ± 0.03      | 0.20 ± 0.03   | 0.27 ± 0.00            | 0.24 ± 0.04   |
| <b>Tb.N. (1/mm)</b> | 0.53 ± 0.19      | 2.12 ± 0.36   | 1.37 ± 0.07            | 1.78 ± 0.23   |
| <b>Tb.Sp. (mm)</b>  | 0.45 ± 0.06      | 0.33 ± 0.09   | 0.49 ± 0.08            | 0.34 ± 0.07   |

**Supplemental Table S2.** Bioreactor-generated tissue scoring guide.

| <b>Category</b>                            | <b>Score</b> | <b>Description</b>  |
|--|--------------|---|
| <b>Tissue Type</b>                         |              |   |
|  | 0            | Significant presence of inflammatory cells  |
|  | 1            | Primarily fibrovascular tissue  |
|  | 2            | Presence of any amount of viable bone tissue                                      |
| <b>Tissue Maturity</b>                     |              |   |
|  | 0            | No viable bone tissue   |
|  | 1            | Presence of any viable osteoid  |
|  | 2            | Presence of any viable woven bone   |
|  | 3            | Presence of any viable lamellar bone  |
| <b>Osteoclast Presence</b>                 |              |   |
|  | 0            | No osteoclast-like cells observed   |
|  | 1            | Osteoclast-like cells observed  |
| <b>Residual Synthetic Graft Intactness</b> |              |   |
|  | 0            | No synthetic scaffold present   |
|  | 1            | Synthetic scaffold shows signs of degradation; no viable bone tissue              |
|  | 2            | Synthetic scaffold intact; no viable bone tissue                                  |
|  | 3            | Synthetic scaffold intact; viable bone tissue                                     |
|  | 4            | Synthetic scaffold shows signs of degradation; viable bone tissue                 |
| <b>Substrate Score</b>                     |              |   |
|  | 0            | No scaffold present   |
|  | 1            | Scaffold present; no viable bone tissue   |
|  | 2            | Scaffold present; viable bone tissue on <25% of available scaffold surface area   |
|  | 3            | Scaffold present; viable bone tissue on 25-75% of available scaffold surface area |
|  | 4            | Scaffold present; viable bone tissue on >75% of available scaffold surface area   |

**Supplemental Table S3.** Bioreactor-generated tissue histomorphometric analysis.

| <b>Assay</b>                   | <b>Autograft</b> | <b>Synthetic Graft</b> |
|--------------------------------|------------------|------------------------|
| <b>Height of Viable Tissue</b> | 0.58 ± 0.31      | 0.58 ± 0.19            |
| <b>Tissue Composition</b>      |                  |                        |
| RSG (%)                        | 2.82 ± 0.57      | 13.51 ± 1.67           |
| Bone (%)                       | 3.96 ± 2.67      | 28.6 ± 3.57            |
| RSG + Bone (%)                 | 39.78 ± 7.57     | 42.22 ± 10.20          |

**Supplemental Table S4.** Bioreactor-generated tissue histologic scoring analysis. Numbers refer to percentage of specimens with given score. Refer to Supplemental Table S2 for explanation of scores. Scores which do not apply to specific categories have been labeled “NA”.

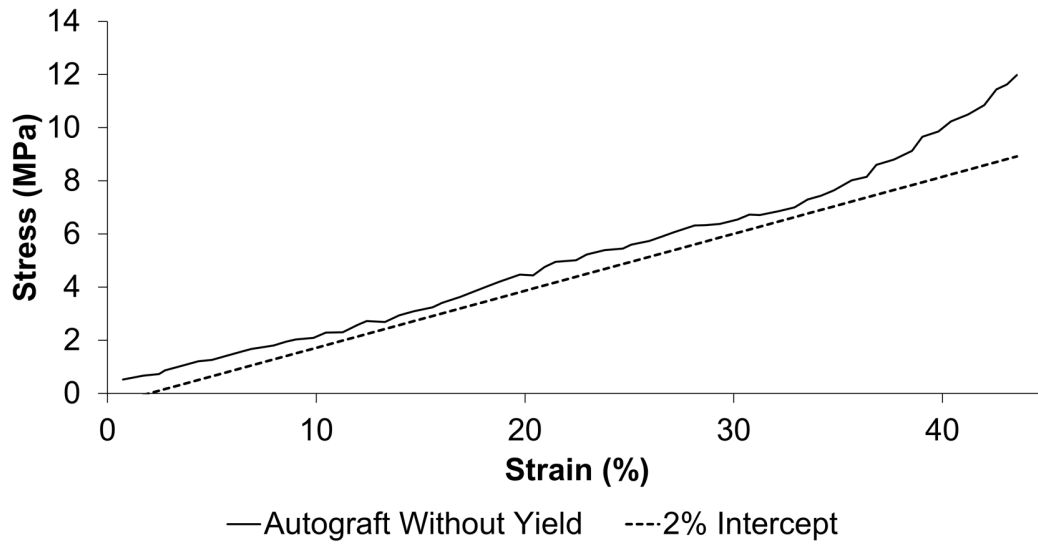
| <b>Category</b>            | <b>Autograft</b> |          |          |          |          | <b>Synthetic Graft</b> |          |          |          |          |
|----------------------------|------------------|----------|----------|----------|----------|------------------------|----------|----------|----------|----------|
| <b>Score</b>               | <b>0</b>         | <b>1</b> | <b>2</b> | <b>3</b> | <b>4</b> | <b>0</b>               | <b>1</b> | <b>2</b> | <b>3</b> | <b>4</b> |
| <b>Tissue Type</b>         | 0                | 0        | 100      | NA       | NA       | 0                      | 0        | 100      | NA       | NA       |
| <b>Tissue Maturity</b>     | 0                | 0        | 0        | 100      | NA       | 0                      | 0        | 37.5     | 62.5     | NA       |
| <b>Osteoclast Presence</b> | 0                | 100      | NA       | NA       | NA       | 87.5                   | 12.5     | NA       | NA       | NA       |
| <b>RSG Intactness</b>      | 100              | 0        | 0        | 0        | 0        | 0                      | 0        | 0        | 0        | 100      |
| <b>Substrate Score</b>     | 0                | 0        | 0        | 0        | 100      | 0                      | 0        | 37.5     | 25       | 37.5     |

**Supplemental Table S5.** Mandible histological scoring guide.

| <b>Category</b>                  | <b>Score</b> | <b>Description</b>   |
|----------------------------------|--------------|--|
| <b>Bony Bridging</b>             |              |  |
|                                  | 0            | No presence of any transferred tissue                          |
|                                  | 1            | <25% bridging of defect with bony tissue                       |
|                                  | 2            | 25%-75% bridging of defect with bony tissue                    |
|                                  | 3            | >75% bridging of defect with bony tissue                       |
| <b>Hardware Osteointegration</b> |              |  |
|                                  | 0            | Screw present; majority of surrounding tissue is fibrovascular |
|                                  | 1            | Screw present; partly integrated in osseous tissue             |
|                                  | 2            | Screw present; fully integrated in osseous tissue              |
| <b>Periosteal Reaction</b>       |              |  |
|                                  | 0            | No presence of any periosteal bone formation                   |
|                                  | 1            | Presence of periosteal bone formation                          |

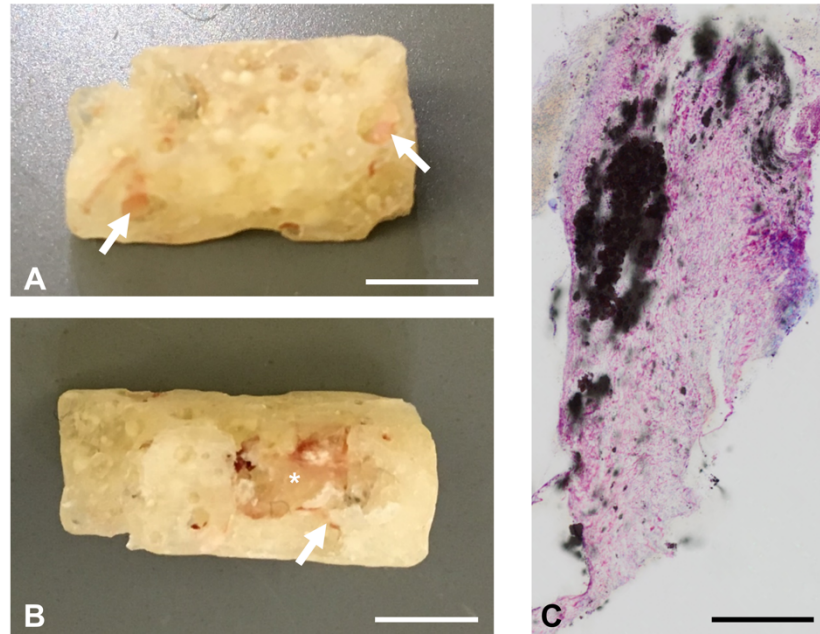
**Supplemental Table S6.** Angiogenic, osteogenic, and chondrogenic genes and ovine-specific primer sequences studied.

| <i>Gene</i>             | <i>Sense</i>           | <i>Antisense</i>           |
|-------------------------|------------------------|----------------------------|
| <i>GAPDH</i> [40]       | GCAAGTTCCACGGCACAG     | GGTTCACGCCCATCACAA         |
| <i>bFGF</i> [41]        | CGACGGCCGAGTGGAC       | CTCTCTTCTGCTTGAAGTTGTAGTTG |
| <i>VEGF</i> [41]        | GGATGTCTACCAGCGCAGC    | TCTGGGTACTCCTGGAAGATGTC    |
| <i>Runx2</i> [40]       | TCGCCTCACAAACAACCA     | AGGGACCTGCGGAGATTA         |
| <i>Osteocalcin</i> [40] | AGATGCAAAGCCTGGTGATGC  | CTCCTGGAAGCCGATGTGGT       |
| <i>BMP2</i> [40]        | ATGGTTTCGTGGTGGAGGTAG  | ACTTGAGGCGTTTCCGCTGTT      |
| <i>BMP7</i> [40]        | AGTCTGACCTGTTCTGCTCG   | GTGGTTGCTGGTGGCTGTG        |
| <i>Sox9</i> [40]        | ATGAAGATGACCGACGAGCAG  | AGTCGTAGCCCTTGAGCACC       |
| <i>Collagen II</i> [42] | CAGGTTACATACACCGTTCTGA | AATCACAGTCTCGCCCCACTT      |

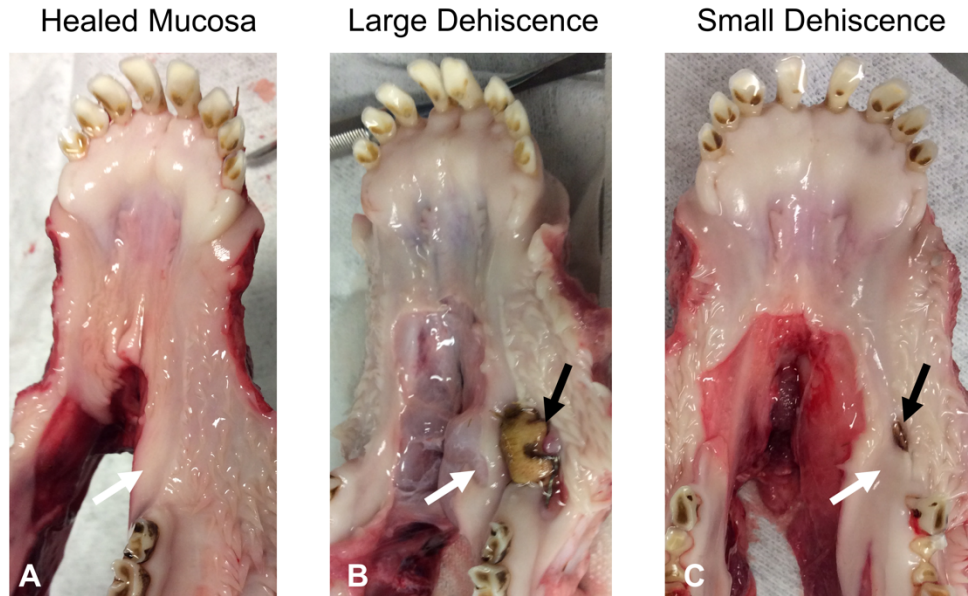


**Supplemental Figure S1.** Example stress/strain curve of one of the two AG specimens that did not fracture nor intersect the 2% offset yield line during unconfined compression testing.

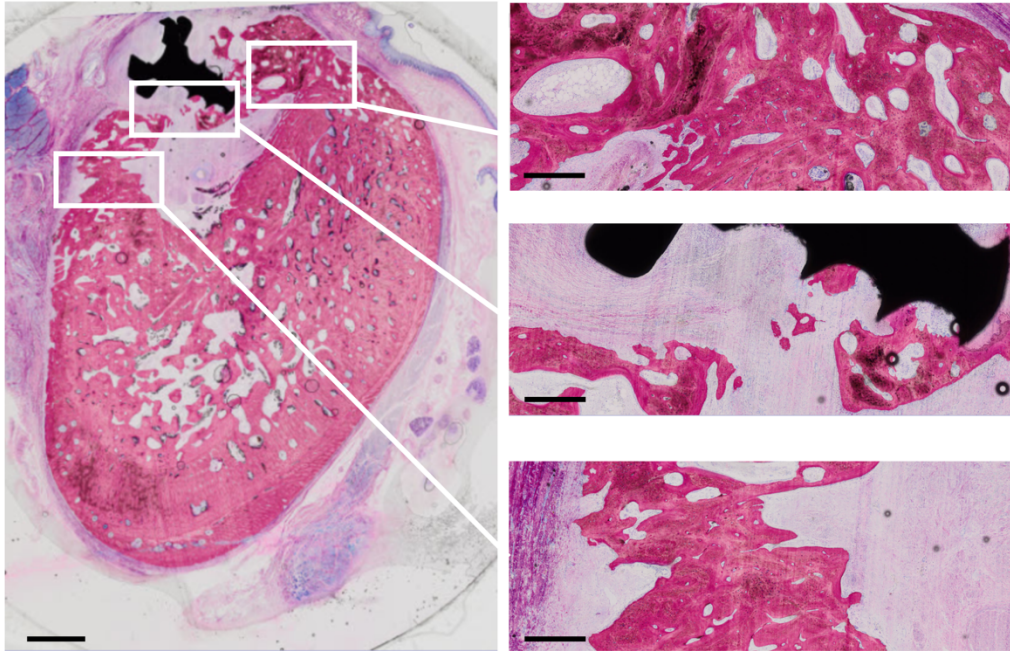




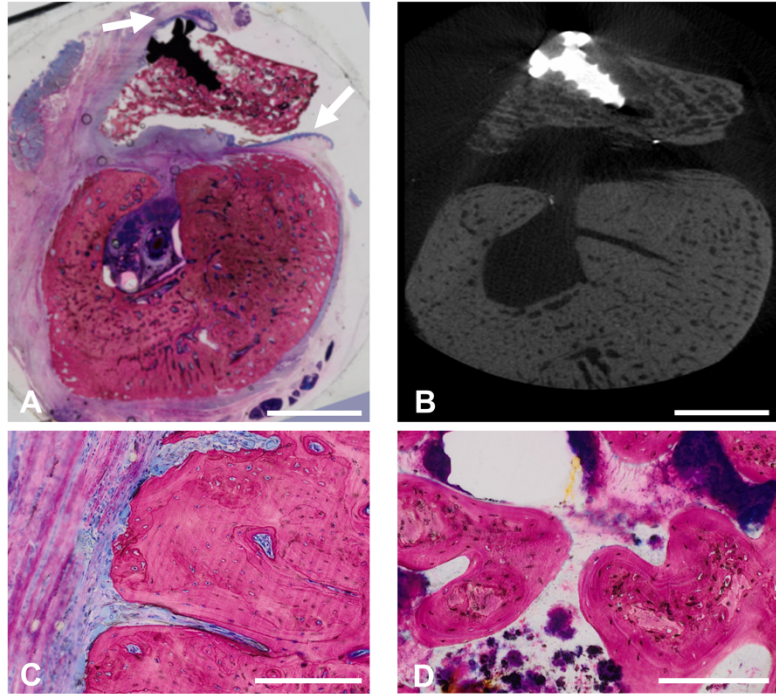
**Supplemental Figure S2.** PBSM photographs. PBSMs were harvested with minimal disruption to the soft tissue envelope. A) Lateral view of a recovered PBSM. Arrows indicate tissue grown into pores. Scale bar = 5 mm. B) Superior view of recovered PBSM. \* indicates patch of tissue grown over the surface of the PBSM. Arrow indicates tissue grown into a pore. Scale bar = 5 mm. C) Histological section of fibrous tissue recovered from PBSM. Scale bar = 200  $\mu$ m.



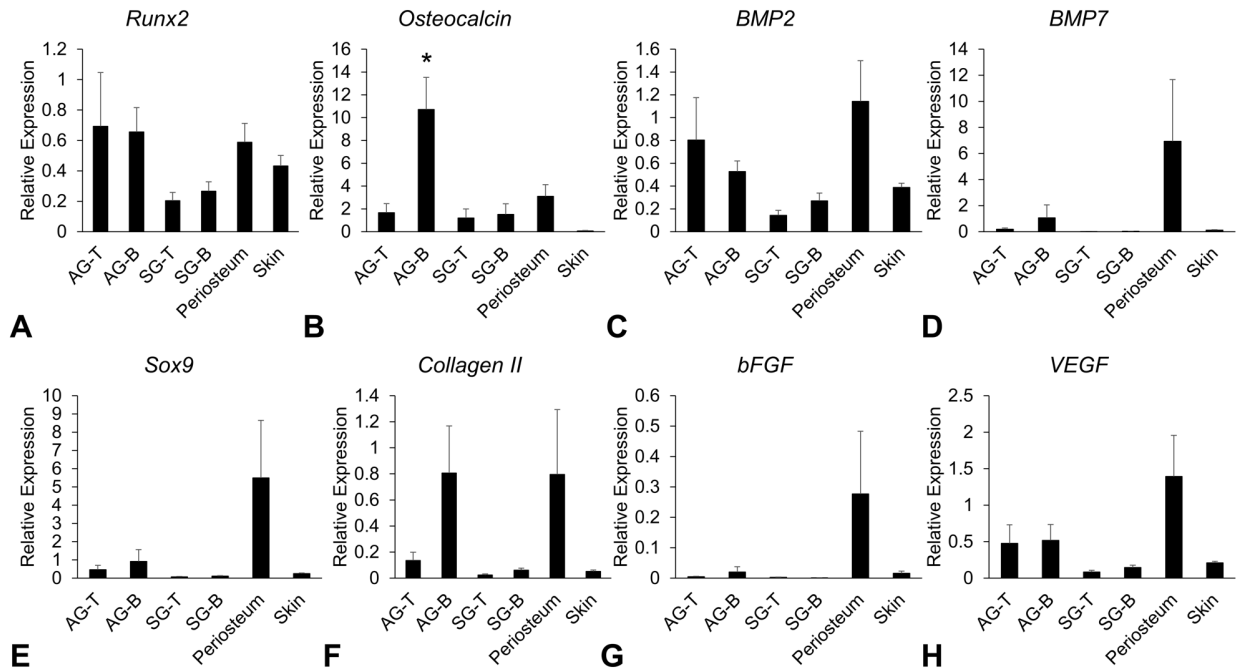
**Supplemental Figure S3.** Superior views of gross harvested mandibles. A) Healed mucosa (appearance of four animals), B) large dehiscence, and C) small dehiscence. White arrow = general region of the reconstructed superior margin. Black arrow = dehiscence of the oral mucosa.



**Supplemental Figure S4.** Example of a reconstructed mandible (left) with high magnification images of different areas (right), demonstrating no visible areas of active inflammation. Areas such as the screw/bone interface and flap/native bone interface were examined and did not show infiltrates of inflammatory cells. Left scale bar = 2 mm and right scale bars all = 0.5 mm.



**Supplemental Figure S5.** Images from the hemi-mandible post-reconstruction of the hemi-mandible with the large dehiscence (Sheep 5). A) Whole cross-section of the diastema. White arrows = mucosa. B) Microcomputed tomography reconstruction. C) High magnification of the edge of an area of periosteal reaction, demonstrating viable osteocytes and new bone deposition. D) High magnification of an area within the flap, demonstrating non-viable osteocytes as well as remaining autograft embedded within bone. Top scale bars = 5 mm and bottom scale bars = 200  $\mu\text{m}$ .



**Supplemental Figure S6.** Gene expression of the soft tissue envelope surrounding bioreactor-generated tissues. A-H) Fold expression relative to *GAPDH* of *Runx2*, *Osteocalcin*, *BMP2*, *BMP7*, *Sox9*, *Collagen II*, *bFGF*, and *VEGF*. \* indicates statistical significance ( $p < 0.05$ ) compared to all other groups. Error bars represent standard error of the mean. AG-T n = 6, AG-B n = 6, SG-T n = 8, SG-B n = 8, Skin n = 18, and Periosteum n = 18 for all genes, except *Runx2* for which Periosteum n = 16.