## Supplementary Information



Figure S1. Specifications of the silicon wafer, PDMS replicas, and films. (a) Design of the microchannels (MCs) features on the silicon wafer. (b) PDMS was manufactured, and it had a negative copy of the MCs on its surface. Dimensions of the PDMS pieces were shown (c) for single and (d) combined layout. Dimensions of the collagen/silk fibroin films and their patterned regions. (e) 2D scaffold. (f) Long film strip that was used in 3D scaffold preparation.


Figure S2: PDMS replicas had the negative prints of the MCs. They were prepared for the production of (a) 2D scaffold as the single piece and (b) long film strip as the combined five pieces.

Table S1: Pairwise comparison of RUNX2 expression.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| TCPS | $5 \%-12 \%$ | $*$ |
| TCPS | $5 \%-21 \%$ | $*$ |
| TCPS | $12 \%-21 \%$ | $* * * *$ |
| 2D | $5 \%-21 \%$ | $*$ |
| $3 D$ | $12 \%-21 \%$ | $* *$ |
| $5 \%$ | TCPS -3D | $* * *$ |
| $5 \%$ | $2 D-3 D$ | $* * *$ |
| $12 \%$ | TCPS - 2D | $* * * *$ |
| $12 \%$ | TCPS - 3D | $* * * *$ |
| $12 \%$ | $2 D-3 D$ | $* *$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01, * * * \mathrm{p} \leq 0.001$, and $* * * * \mathrm{p} \leq 0.0001$

Table S2: Pairwise comparison of OSX expression.

| Group | Pairs | Statistical difference |
| :---: | :---: | :---: |
| 3D | 5\%-21\% | **** |
| 3D | 12\%-21\% | **** |
| 5\% | TCPS - 3D | ** |
| 5\% | 2D-3D | ** |
| 12\% | TCPS - 3D | * |
| 12\% | 2D-3D | * |
| 21\% | TCPS - 3D | **** |
| 21\% | 2D-3D | **** |

Table S3: Pairwise comparison of BST1 expression.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| TCPS | $5 \%-21 \%$ | $* *$ |
| 2D | $5 \%-12 \%$ | $* * * *$ |
| 2D | $5 \%-21 \%$ | $* * * *$ |
| $5 \%$ | TCPS -2D | $* * *$ |
| $5 \%$ | $2 D-3 D$ | $* *$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and ${ }^{* * * *} \mathrm{p} \leq 0.0001$

Table S4: Pairwise comparison of CD90 expression.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| TCPS | $5 \%-12 \%$ | $* *$ |
| TCPS | $5 \%-21 \%$ | $* * * *$ |
| TCPS | $12 \%-21 \%$ | $*$ |
| 3D | $5 \%-21 \%$ | $* *$ |
| 3D | $12 \%-21 \%$ | $* * * *$ |
| $5 \%$ | TCPS -3D | $* * * *$ |
| $5 \%$ | 2D -3D | $* * * *$ |
| $12 \%$ | TCPS - 2D | $* *$ |
| $12 \%$ | TCPS - 3D | $* *$ |
| $12 \%$ | 2D - 3D | $* * * *$ |
| $21 \%$ | TCPS - 2D | $* * * *$ |
| $21 \%$ | TCPS - 3D | $* * *$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and $* * * * \mathrm{p} \leq 0.0001$

Table S5: Pairwise comparison of VEGFA expression.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| 3D | $5 \%-12 \%$ | $* * * *$ |
| 3D | $5 \%-21 \%$ | $* * * *$ |
| 3D | $12 \%-21 \%$ | $* * * *$ |
| $5 \%$ | TCPS - 3D | $* * * *$ |
| $5 \%$ | 2D -3D | $* * * *$ |
| $12 \%$ | TCPS -3D | $* * *$ |
| $12 \%$ | 2D - 3D | $* * *$ |
| $21 \%$ | TCPS -3D | $* * * *$ |
| $21 \%$ | 2D -3D | $* * * *$ |

*p $\leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and $* * * * \mathrm{p} \leq 0.0001$
Table S6: Pairwise comparison of UTS values.

| Group | Subgroup | Pairs | Statistical difference |
| :--- | :--- | :--- | :--- |
| 2D | Unseeded | $5 \%-21 \%$ | $* *$ |
| 2D | MSC seeded | $5 \%-21 \%$ | $* *$ |
| 3D | MSC seeded | $5 \%-21 \%$ | $* * * *$ |
| $21 \%$ | MSC seeded | 2D $-3 D$ | $* *$ |
| $21 \%$ | 3D | Unseeded - MSC seeded | $* * * *$ |

[^0]Table S7: Pairwise comparison of $E$ values.

| Group | Subgroup | Pairs | Statistical difference |
| :--- | :--- | :--- | :--- |
| 2D | Unseeded | $5 \%-21 \%$ | $*$ |
| 2D | MSC seeded | $5 \%-21 \%$ | $* * * *$ |
| 3D | MSC seeded | $5 \%-21 \%$ | $* *$ |
| $21 \%$ | 2D | Unseeded - MSC seeded | $*$ |
| $21 \%$ | 3D | Unseeded - MSC seeded | $* *$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and ${ }^{* * * *} \mathrm{p} \leq 0.0001$

Table S8: Pairwise comparison of increase in UTS values.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| 3 D | $5 \%-21 \%$ | $* * * *$ |
| $21 \%$ | $2 \mathrm{D}-3 \mathrm{D}$ | $* * * *$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01, * * * \mathrm{p} \leq 0.001$, and $* * * * \mathrm{p} \leq 0.0001$

Table S9: Pairwise comparison of increase in $E$ values.

| Group | Pairs | Statistical difference |
| :--- | :--- | :--- |
| 3D | $5 \%-21 \%$ | $*$ |

* $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and $* * * * \mathrm{p} \leq 0.0001$

Table S10: Information about the target genes for real-time RT-PCR.

| Gene | NCBI accession no. | Brand | Product code |
| :--- | :--- | :--- | :--- |
| HPRT1 | NG 012329.1 | Biorad | qHsaCID0016375 |
| RUNX2 | NG 008020.1 | Biorad | qHsaCID0006726 |
| BST1 | NC 000004.11 | Biorad | qHsaCID0013947 |
| VEGFA | NG 008732.1 | Biorad | qHsaCED0043454 |
| 18S rRNA | X03205.1 | Qiagen | QT00199367 |
| OSX | NM 152860.1 | Qiagen | QT00213514 |
| CD90 | NM 006288.4 | Qiagen | QT00023569 |


[^0]:    * $\mathrm{p} \leq 0.05,{ }^{* *} \mathrm{p} \leq 0.01,{ }^{* * *} \mathrm{p} \leq 0.001$, and ${ }^{* * * *} \mathrm{p} \leq 0.0001$

