

## **SUPPLEMENTAL INFORMATION**

**Title:** 3D bioprinted material that recapitulates the perivascular bone marrow structure for sustained hematopoietic and cancer models.

**Authors:** Caitlyn A. Moore<sup>a,b</sup>, Zain Siddiqui<sup>c</sup>, Griffin J. Carney<sup>a</sup>, Yahaira Naaldijk<sup>d</sup>, Khadidiatou Guir<sup>b</sup>, Murat Guvendiren, Vivek A. Kumar<sup>c,e,f</sup>, Pranela Rameshwar<sup>a,b\*</sup>

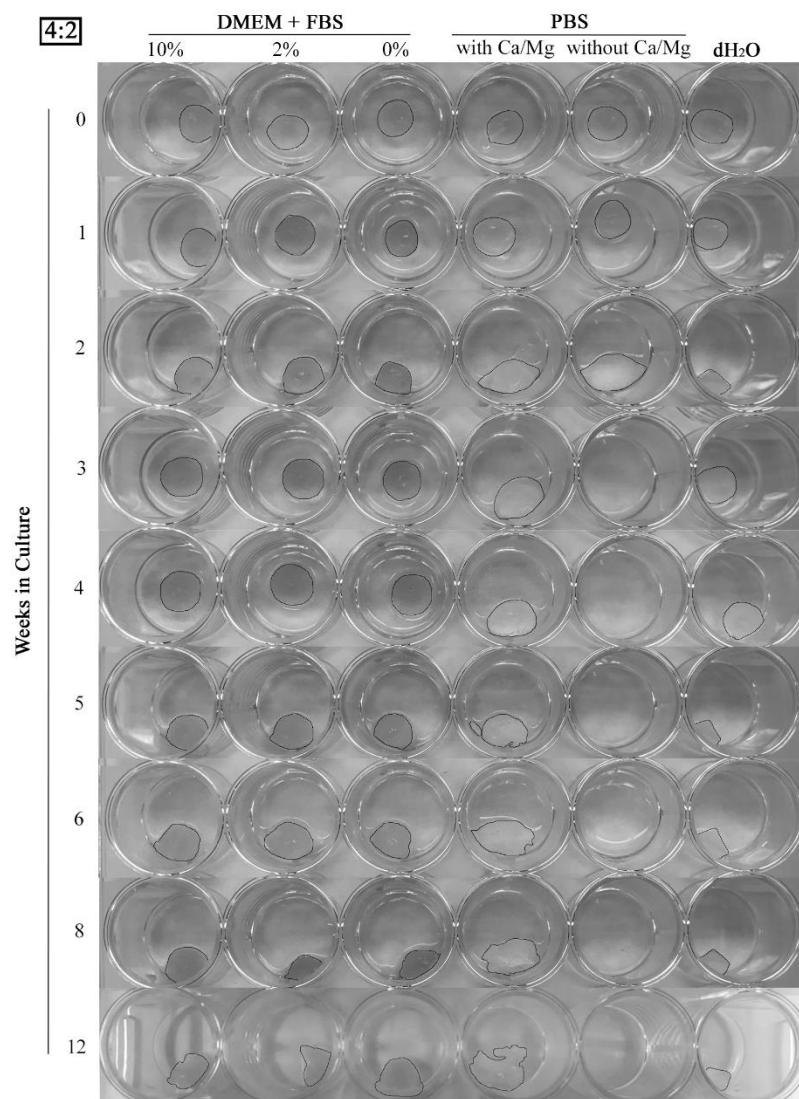
Supplemental Table 1

Bioink Name Designation:			0:2	1:2	2:2	4:0	4:1	4:2	6:0	6:1	6:2
Bioink Components (% w/v)		MC	0	1	2	4	4	4	6	6	6
		Alginate	2	2	2	0	1	2	0	1	2
Performance Criteria	Rank	Weight									
Extrudability	1	35%	0.00	0.35	0.35	0.35	0.70	1.05	0.35	0.35	0.70
Handleability	2	30%	0.00	0.00	0.00	0.00	0.30	0.90	0.00	0.30	0.90
Resolution and Fidelity	3	20%	0.00	0.00	0.00	0.20	0.40	0.40	0.20	0.40	0.40
Ease of Fabrication	4	15%	0.45	0.45	0.45	0.45	0.45	0.45	0.30	0.15	0.15
Overall Performance:			0.45	0.80	0.80	1.00	1.85	2.80	0.85	1.20	2.15



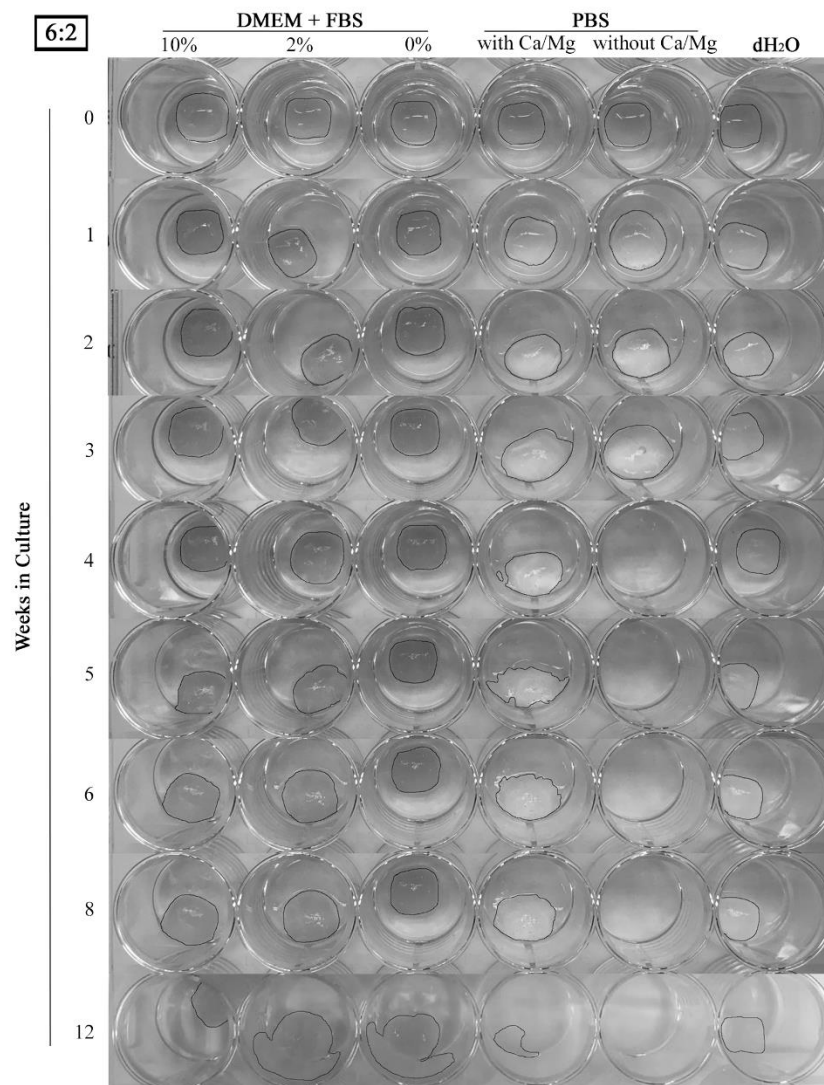
**Table S1:** A decision matrix was established to identify potential candidate methylcellulose (MC)-alginate bioink formulations. Bioinks were grouped based on low (**green**), medium (**red**), or high (**blue**) relative MC content, and compared using 4 arbitrarily-weighted performance criteria: extrudability, handleability, resolution and fidelity, and ease of fabrication. Performance of bioinks across these criteria was scored on a scale of 0 (**poor**) to 3 (**excellent**) and totaled to provide an overall performance score. Bioinks with the highest performance scores (**black arrows**) were considered to be most feasible for use and were selected for further study.

Supplemental Fig. 1



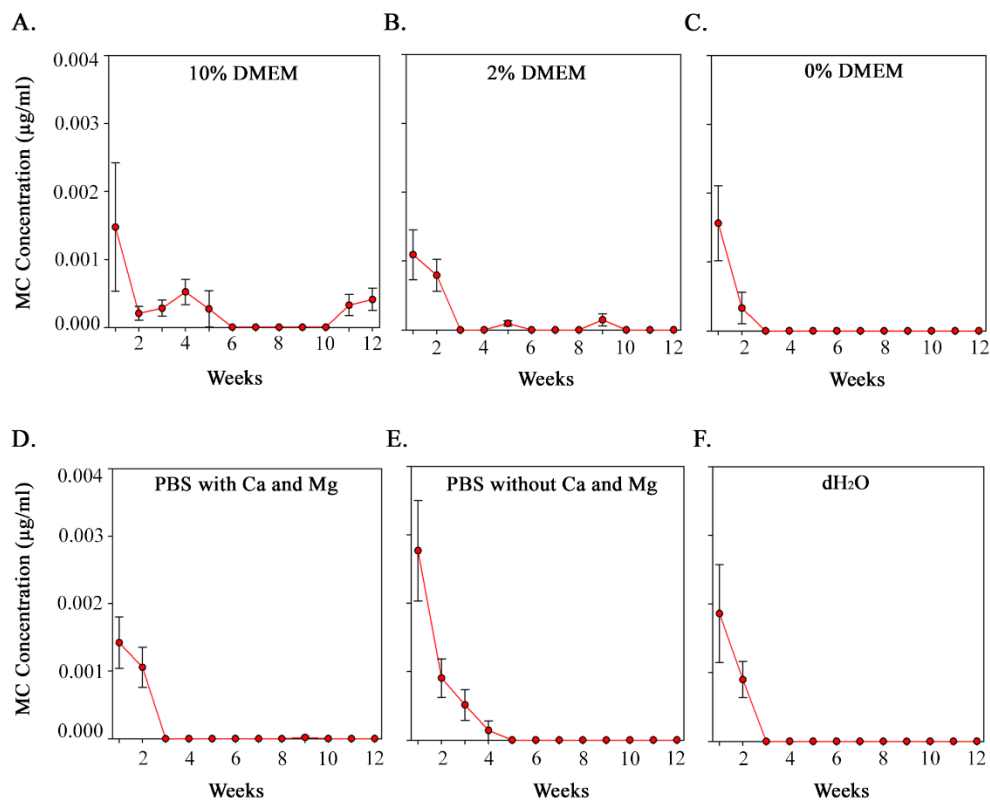
**Figure S1. Visible degradation of 4:2 scaffolds in culture solutions.** Images of 4:2 scaffolds were acquired weekly for 12 weeks (labeled left; weeks 7, 9, 10, 11 omitted). Scaffolds were cultured in DMEM with 10%, 2%, or 0% FBS, PBS with and without Ca<sup>2+</sup> and Mg<sup>2+</sup>, and deionized water (dH<sub>2</sub>O) (labeled top).

Supplemental Fig. 2



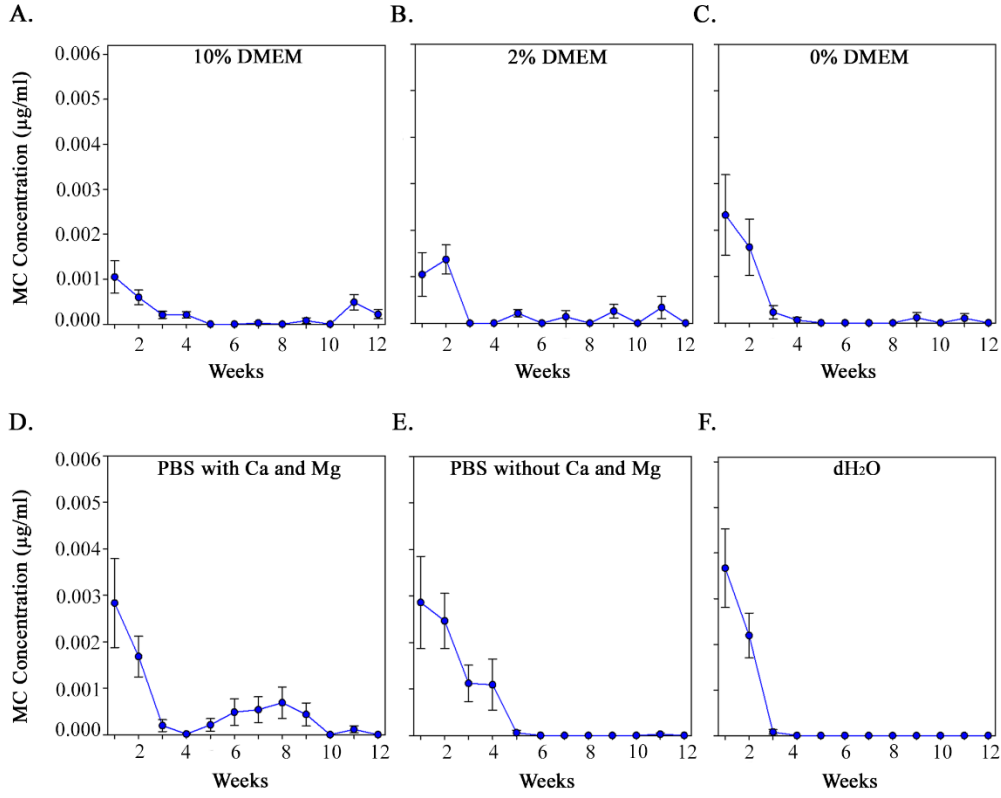
**Figure S2. Visible degradation of 6:2 scaffolds in culture solutions.** Images of 6:2 scaffolds were acquired weekly for 12 weeks (labeled left; weeks 7, 9, 10, 11 omitted). Scaffolds were cultured in DMEM with 10%, 2%, or 0% FBS, PBS with and without Ca<sup>2+</sup> and Mg<sup>2+</sup>, and deionized water (dH<sub>2</sub>O) (labeled top).

Supplemental Fig. 3



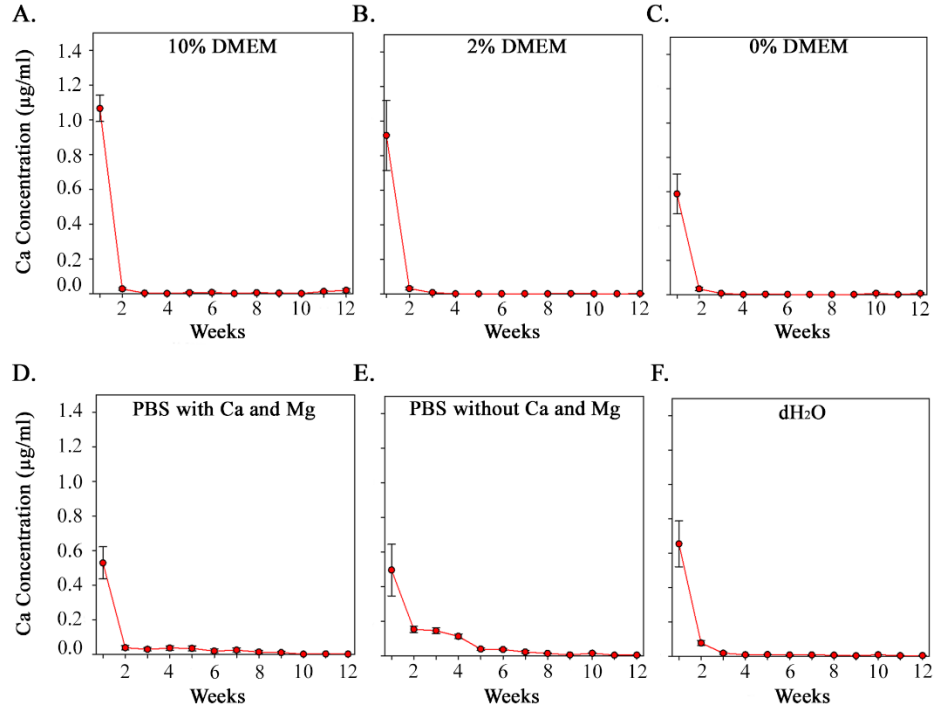
**Figure S3. MC released from 4:2 scaffolds during long-term culture.** The concentration of MC released from 4:2 scaffolds was measured weekly in culture solutions: DMEM containing (A) 10% FBS, (B) 2% FBS, or (C) 0% FBS, (D) PBS with Ca<sup>2+</sup> and Mg<sup>2+</sup>, (E) PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>, and (F) deionized water (dH<sub>2</sub>O). Data represents n=3 with at least 4 technical replicates.

Supplemental Fig. 4



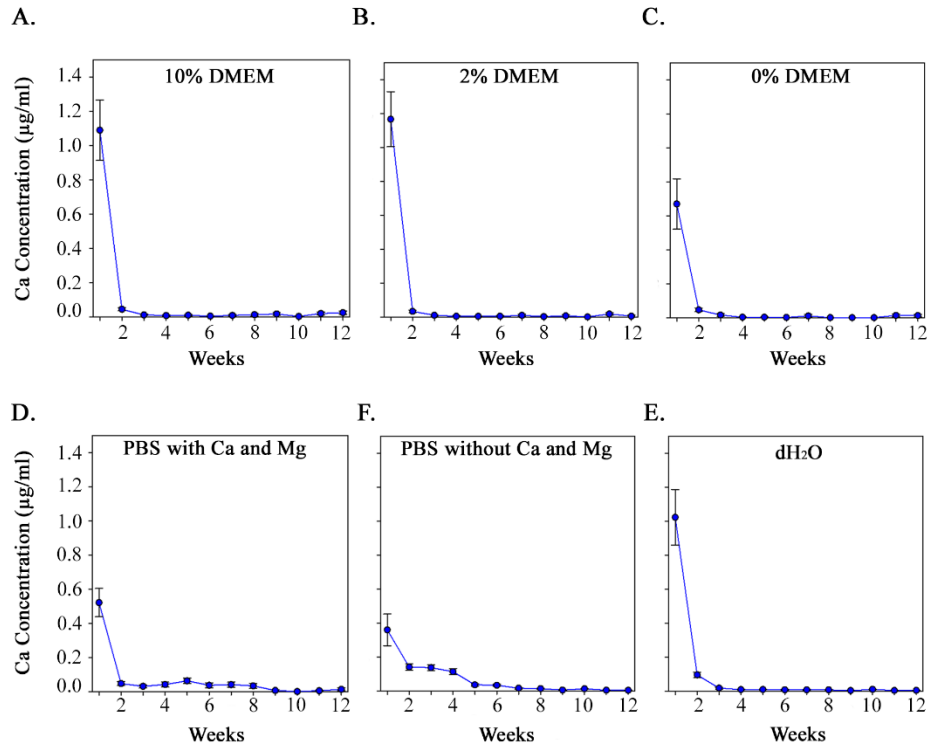
**Figure S4. MC released from 6:2 scaffolds during long-term culture.** The concentration of MC released from 4:2 scaffolds was measured weekly in culture solutions: DMEM containing (A) 10% FBS, (B) 2% FBS, or (C) 0% FBS, (D) PBS with Ca<sup>2+</sup> and Mg<sup>2+</sup>, (E) PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>, and (F) deionized water (dH<sub>2</sub>O). Data represents n=3 with at least 4 technical replicates.

Supplemental Fig. 5



**Figure S5. Alginate crosslink breakage in 4:2 scaffolds during long-term culture.**  $\text{Ca}^{2+}$  in solution served as an indicator for alginate crosslink breakage in 4:2 scaffolds cultured in various solutions: DMEM containing (A) 10% FBS, (B) 2% FBS, or (C) 0% FBS, (D) PBS with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , (E) PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and (F) deionized water ( $\text{dH}_2\text{O}$ ). Data represents  $n=3$  with at least 4 technical replicates.

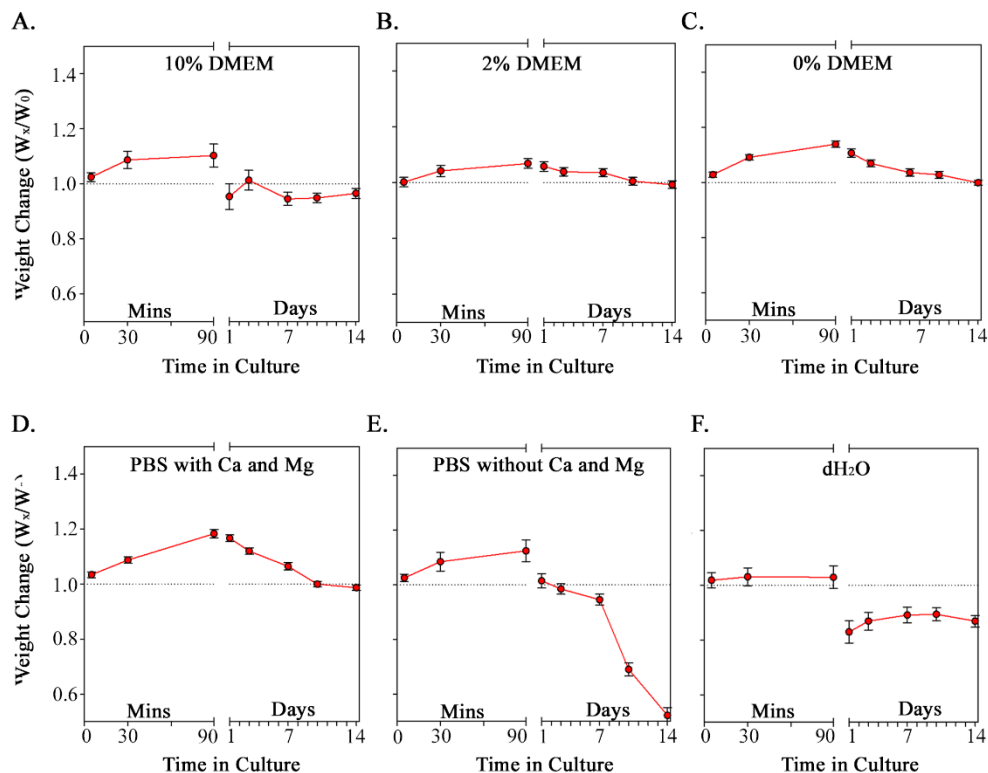
Supplemental Fig. 6



**Figure S6. Alginate crosslink breakage in 6:2 scaffolds during long-term culture.**  $\text{Ca}^{2+}$  in solution served as an indicator for alginate crosslink breakage in 4:2 scaffolds cultured in various solutions: DMEM containing (A) 10% FBS, (B) 2% FBS, or (C) 0% FBS, (D) PBS with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , (E) PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and (F) deionized water ( $\text{dH}_2\text{O}$ ). Data represents  $n=3$  with at least 4 technical replicates.

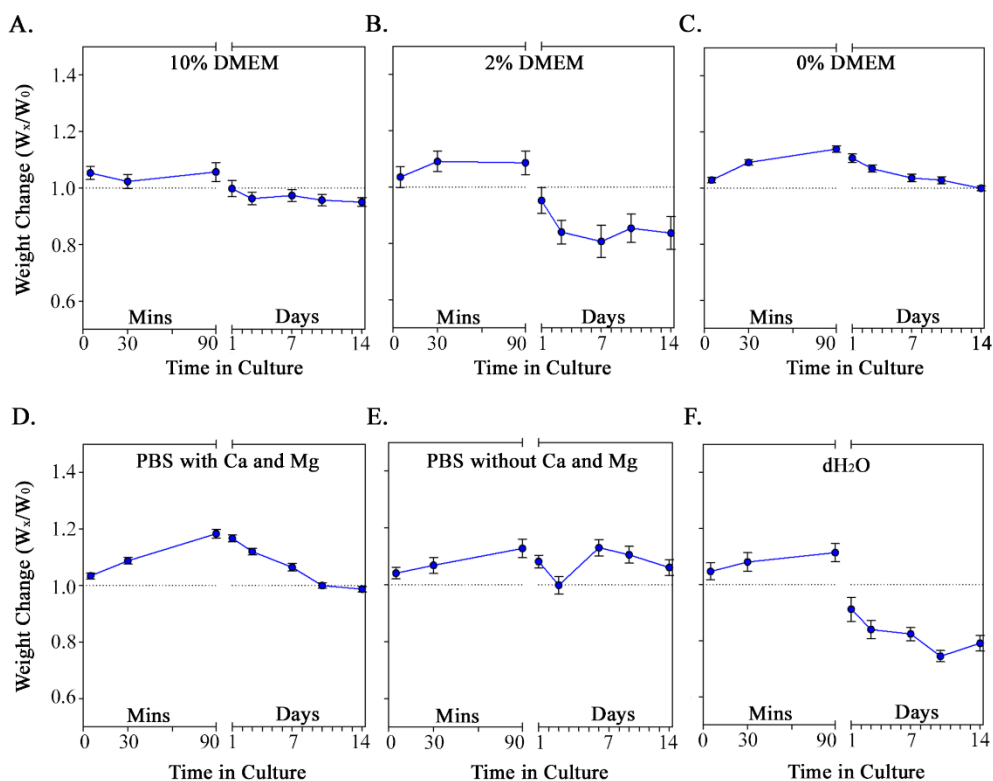


Supplemental Fig. 7



**Figure S7. Weight fluctuation of 4:2 scaffolds during the first two weeks of culture.** Weight of 4:2 scaffolds was measured at various times after printing ( $W_x$ ) and normalized by initial weight (immediately after crosslinking,  $W_0$ ) to yield a weight change ratio ( $W_x/W_0$ ) for each scaffold. Scaffolds were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing (A) 10%, (B) 2%, or (C) 0% fetal bovine serum (FBS), (D) PBS with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , (E) PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and (F) deionized water ( $\text{dH}_2\text{O}$ ). Dotted line indicates initial weight ( $W_0/W_0$ ). Data represents  $n=4$  with at least 3 technical replicates.

Supplemental Fig. 8



**Figure S8. Weight fluctuation of 6:2 scaffolds during the first two weeks of culture.** Weight of 4:2 scaffolds was measured at various times after printing ( $W_x$ ) and normalized by initial weight (immediately after crosslinking,  $W_0$ ) to yield a weight change ratio ( $W_x/W_0$ ) for each scaffold. Scaffolds were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing (A) 10%, (B) 2%, or (C) 0% fetal bovine serum (FBS), (D) PBS with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , (E) PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and (F) deionized water ( $\text{dH}_2\text{O}$ ). Dotted line indicates initial weight ( $W_0/W_0$ ). Data represents  $n=4$  with at least 3 technical replicates.