

1 Supporting Information

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3 **Construction of Skeletal Muscle Tissue based on Edible Blood-Derived**
4 **Components using a PDMS Substrate and 3D-Printed Anchors**

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Sup 1: Sensory evaluation questionnaire (English translation)

*Original questionnaire was described in Japanese

Sensory Evaluation Questionnaire for Cultured Meat

No.

【Sensory Evaluation of Cultured Meat】

Please circle the number that best represents your evaluation for each attribute.
In the comment section, feel free to write the reasons for your evaluation and any impressions.

*Notes for Evaluation

- Please evaluate objectively, without including personal preferences.
- If there is a discrepancy between your objective evaluation and personal preference, please describe it in the comment section.
- Please rate conventional beef as “3” on the scale.

(1) Texture Evaluation

Firmness during mastication (Perceived firmness when biting)

No.	Comment (reason for rating, impression, etc.)	Rating				
		Very hard ←————→ Very soft				
		1	2	3	4	5
1						
2						

Fibrous texture (Perception of muscle fiber structure during chewing)

No.	Comment (reason for rating, impression, etc.)	Rating				
		Very weak ←————→ Very strong				
		1	2	3	4	5
1						
2						

Juiciness (Perceived juiciness during chewing)

No.	Comment (reason for rating, impression, etc.)	Rating				
		Very weak ←————→ Very strong				
		1	2	3	4	5
1						
2						

(2) Taste evaluation

Sweetness

No.	Comment (reason for rating, impression, etc.)	Rating				
		Very weak ←————→ Very strong				
		1	2	3	4	5
1						
2						

1 Sourness

No.	Comment (reason for rating, impression, etc.)	Rating					
		Very weak		↔			Very strong
		1	2	3	4	5	
1							
2							

2

3 Bitterness

No.	Comment (reason for rating, impression, etc.)	Rating					
		Very weak		↔			Very strong
		1	2	3	4	5	
1							
2							

4

5 Umami

No.	Comment (reason for rating, impression, etc.)	Rating				
		Very weak ↔ Very strong				
		1	2	3	4	5
1						
2						

6

7 (3) Aroma evaluation

8 Fatty aroma (Perceived aroma retronasally, e.g., butter, salad oil)

No.	Comment (reason for rating, impression, etc.)	Rating					
		Very weak		↔			Very strong
		1	2	3	4	5	
1							
2							

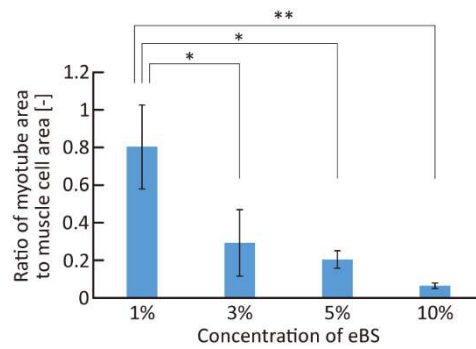
9

10 Meaty aroma (Perceived aroma retronasally, e.g., beef jerky, corned beef, beef extract)

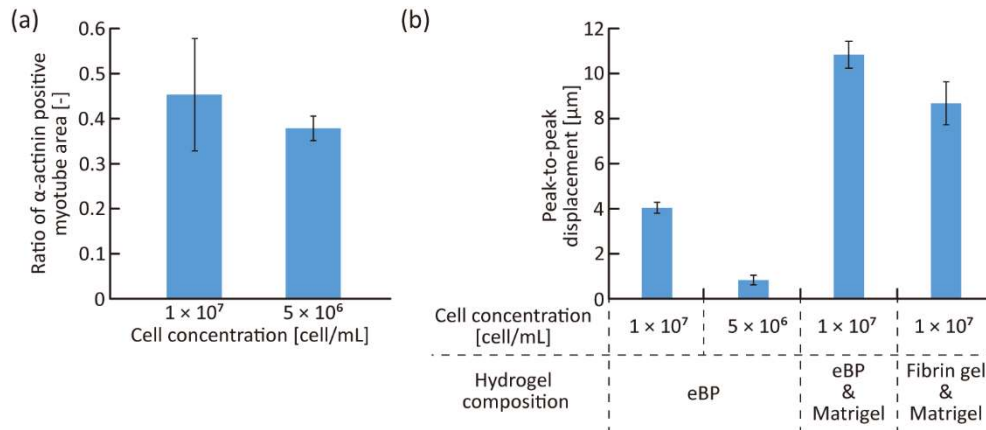
No.	Comment (reason for rating, impression, etc.)	Rating				
		Very weak  Very strong				
		1	2	3	4	5
1						
2						

11

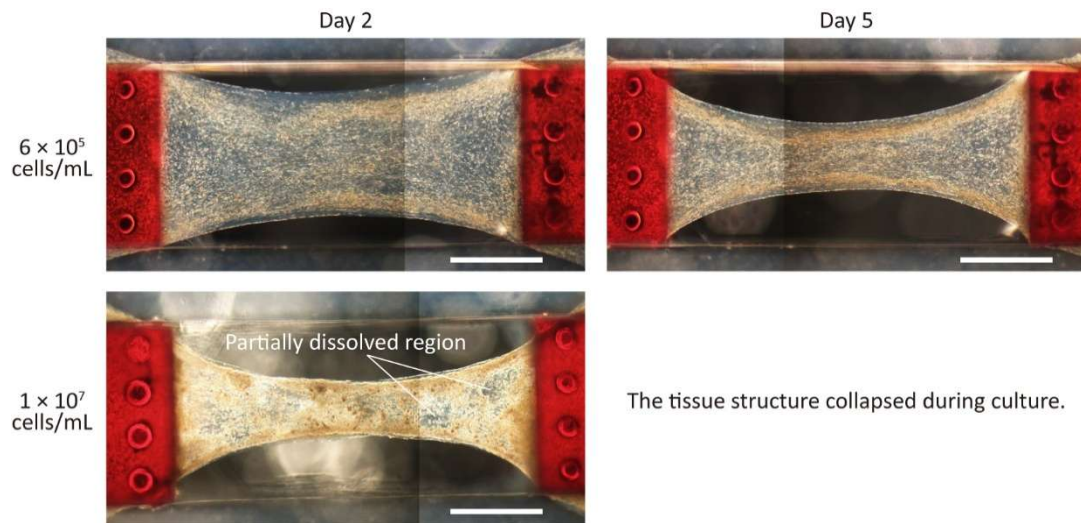
12 Thank you very much for your cooperation.



Sup. Fig. 1. Ratio of myotube area to myocyte area when culturing bovine myocytes on culture dishes (N = 3 samples). Significance was determined with the Tukey's post hoc test (*P < 0.05; **P < 0.01). The myotube and myocyte areas were quantified from bright-field images using ImageJ.



Sup. Fig. 2. Differentiation in cultured skeletal muscle tissue formed using blood components. (a) Quantitative analysis of α -actinin-positive myotube area fraction obtained from immunofluorescence images ($n = 4$ regions) of the bovine skeletal muscle tissue formed by culturing bovine myocyte-laden eBP constructs at 1×10^7 cells/mL and 5×10^6 cells/mL in IMEM supplemented with eBS. The area fraction was calculated using ImageJ after image binarization and segmentation. (b) Comparison of peak-to-peak displacement during single contraction ($n = 7$ contractions) under different cell concentrations and hydrogel compositions: eBP, eBP with Matrigel, and a conventional mixture of fibrin gel and Matrigel (non-edible materials commonly used for skeletal muscle tissue construction). Skeletal muscle tissues composed of fibrin gel and Matrigel were fabricated using a modified version of our previous method [Ren, et al., 2025]. Briefly, after placing the anchors on the PDMS substrate, a hydrogel solution consisting of Matrigel, the growth medium, fibrinogen, thrombin, and bovine myocytes (1×10^7 cells/mL) was gelled on the substrate and cultured for 5 days in the culture medium supplemented with 1% ACA.



Sup. Fig. 3. Images of skeletal muscle tissues formed from bovine myocyte-laden eBP under ACA-free conditions. At a higher cell concentration (1×10^7 cells/mL), constructs exhibited structural degradation and collapse during culture under ACA-free conditions. In contrast, at a lower concentration (6×10^5 cells/mL), the tissue maintained structural integrity. Scale bars are 1 mm.