ISSN: 2155-9627
Journal of Clinical Research & Bioethics

OPEN ACCESS Freely available online

Research Article

Generation of Cultured Beef from Bovine Embryonic Stem Cells

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Supplementary Table 1: Detailed information of antibodies used for staining.

Antibody	Catalogue number	Changling
Live/Dead staining antibody	Alexa Fluor® 488 anti-human SSEA-4	BioLegend 330411
Primary antibody	Mouse anti-Oct3/4 Antibody (C-10)	Santa C1uz sc-5279
	Rat anti-SOX2 Monoclonal Antibody (Btjce)	eBioscience 14-9811-82
	Rabbit anti-Nanog Polyclonal Antibody	PeproTech 500-P236
	Mouse anti-Myosin 4 Monoclonal Antibody (MF20)	eBioscience 14-6503-82
	TRITC-phalloidin TRITC	MKbio MX4405
	BODIPY	MKbio MX5403
Secondary antibody	Alexa Fluor™ 488 Donkey anti-Rabbit IgG (H+L)	Invitrogen A21206
	Alexa Fluor™ 555 Donkey anti-Rabbit IgG (H+L)	Invitrogen A31572
	Alexa Fluor™ 488 Donkey anti-Mouse IgG (H+L)	Invitrogen A21202
	Alexa Fluor™ 555 Donkey anti-Mouse IgG (H+L)	Invitrogen A31570
	Alexa Fluor™ 488 Donkey anti-Rat lgG (H+L)	Invitrogen A21208

Supplementary Table 2: Primer sequences used for qRT-PCR.

Gene	Forward primer	Reverse primer
OCT4	AACGAGAATCTGCAGGAGATATG	TCTCACTCGGTTCTCGATACT
NANOG	TTCCTCCACCCCTTTTAGCC	TGTACTTCAACAAACCAGCCA
SOX2	TGCTGCCTCTTTAAGACTAGGAC	AAATCAGGCGAAGAATAATTTGG
PAX6	GTCTGTACCAACGATAACATACC	GCCTCATCTGAATCTTCTCC
MEOX1	GGAGAATTCAGACAACCAGGAG	TGAGCAAACTCAGCTTCGAG
ALB	ACCAGGAAAGTACCCCAAGTG	GTTTCAACAGCTCAACAAGTGC
SST	CCTGGAGCCTGAAGATTTGTC	GTGAGAAGGGGTTTGGAGAAG
ZIC1	TCTGCTTCTGGGAGGAGTGT	GTGCGTCCTTTTGTGGATCT

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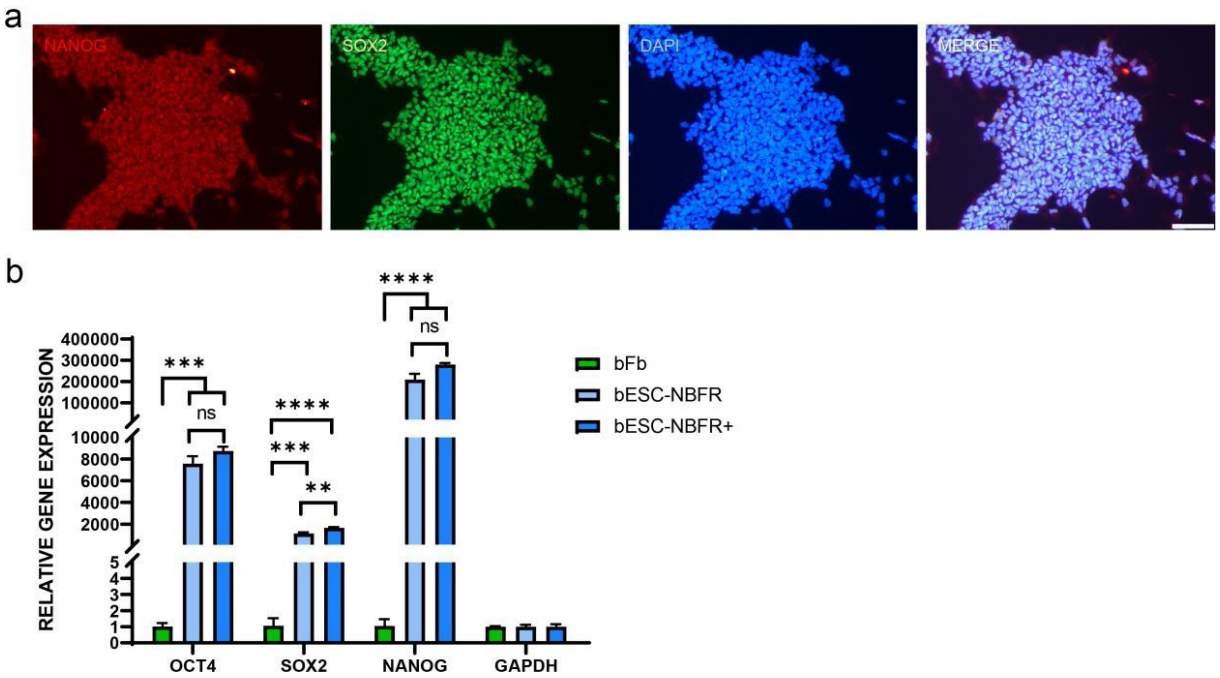
Received: 12-Nov-2024, Manuscript No. JCRB-24-27497; **Editor assigned:** 14-Nov-2024, PreQC No. JCRB-24-27497 (PQ); **Reviewed:** 28-Nov-2024, QC No. JCRB-24-27497; **Revised:** 05-Dec-2024, Manuscript No. JCRB-24-27497 (R); **Published:** 12-Dec-2024, DOI: 10.35248/2155-9627.24.15.504

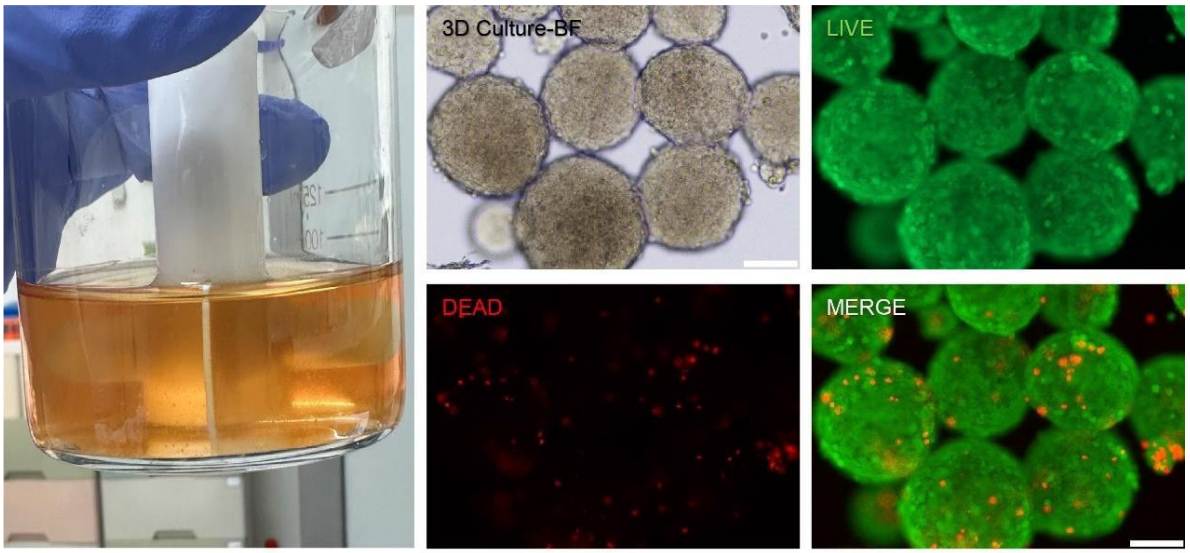
Citation: Rui X, Li Z, Xu J, Dai J, Zhang X, Jin X, et al. (2024). Generation of Cultured Beef from Bovine Embryonic Stem Cells. J Clin Res Bioeth. 15:504.

P75	TGGACAGCGTGACCTTCTC	TCGTCTCGTCCTGGTAATAGC
EIF3K	CTGGCTGAGATGCTCGGGG	CCACGATGTTCTTGGGCTTTAT
ITGAS	CTCTGTGGCTGTGGGTGAAT	GTAGGAGGCCATCTGTTCCC
CD90	CAGAATACAGCTCCCGAACCAA	CACGTGTAGATCCCCTCATCCTT
CD105	CGGACAGTGACCGTGAAGTTG	TGTTGTGGTTGGCCTCGATTA
PPARG	AGACGACAGACAAATCACCGTT	TTCCACGGAGCGAAACTGAC
DLK1	CTTGCTCCTGCTGGCTTTCG	AGGTCACGCACTGGTCACAC
FABP4	GGATGGAAAATCAACCACCA	TGGACAACGTATCCAGCAGA
UCP1	TGCGTGGCTGACATAATCACCTTC	GGCACTGGAGATCAGGCATTCG
PGC1α	AGGCAGAGGCAGAAGGCAATTAAC	CCTCAGTTCTGTCCGTGTTGTGTC
CIDEA	CCTTCCGTGTCTCCAACCATGAC	GCGACCACCAGTGCATCCAAG
ADIPOQ	GGCTCTGATTCCACACCTGA	TGTTGTCCTCGCCATGACTG
T	CACACGGCTGCGAAAGGTA	TGAACTGTCGGAATAGGTTGGA
Pax3	AGGAAGGAGGCAGAGGAGAG	AAGCTGTTCTGCTGTGAAGG
Myogenin	GCGCAGACTCAAGAAGGTGA	TGCAGGCGCTCTATGTACTG
Myoglobin	AGTCAGTCCGCCCTTGTTCT	GGATGACCTGTGAAGAGCCTGA
Desmin	GGAAGCCGAGGAATGGTACA	TCGATCTCGCAGGTGTAGGA
MyoD	TTTGCCAGAGCAGGAGCCCCTC	TTCGAACACCTGAGCGAGCGC
CAV3	GATCGATCTGGTGAACCGGG	TGTAGCTCACCTTCCACACG
MyHC	TGCTCATCTCACCAAGTTCC	CACTCTTCACTCTCATGGACC
MYOSIN	CGACAAGATCGAGGACATGG	AGATGGAGAAGATGTGGGGC
GAPDH	TGACCCCTTCATTGACCTTCA	ACCCCAGTGGACTCCACCACAT

Supplementary Figure 1: A schematic illustration elucidating the mechanical elongation method employed in the fabrication of textured protein scaffolds as described [1]. with modification. Briefly, the formulation process involved the meticulous hand-mixing of soy, gluten, and deionized water at room temperature to achieve a homogeneous dough. Subsequently, the dough underwent a detailed incubation process in a water bath at 60°C for one hour, ensuring comprehensive hydration of the gluten component. Following this hydration phase, the dough was methodically torn into pieces. It was postulated that an additional sequence of stretching and pulling would align the protein network, thereby facilitating protein agglomeration and engendering the formation of robust networks. Conforming to this hypothesis, the doughs were subjected to elongation, reaching twice their initial length, and were then meticulously folded to attain the ultimate dough structure. Each resulting dough was carefully encased in baking paper and subsequently covered with a layer of aluminum foil before undergoing a 30 min steaming procedure. The dough was then methodically sectioned into diminutive pieces and judiciously stored at 4°C for prospective utilization.



Supplementary Figure 2: (a) Immunofluorescence staining of pluripotency markers (NANOG, SOX2) on feeder-free bESCs, cultured in NBFR+ medium. Scale bars equal 100 μm; (b) Gene expression analysis of pluripotency markers (OCT4, SOX2, NANOG) in bESC on feeder in NBFR, and feeder-free bESCs in NBFR+, compared to negative control bFb. (n=3). **Note:** Data are expressed as mean plus standard error of the mean. p-values are labelled: ns (No Significance) indicates $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



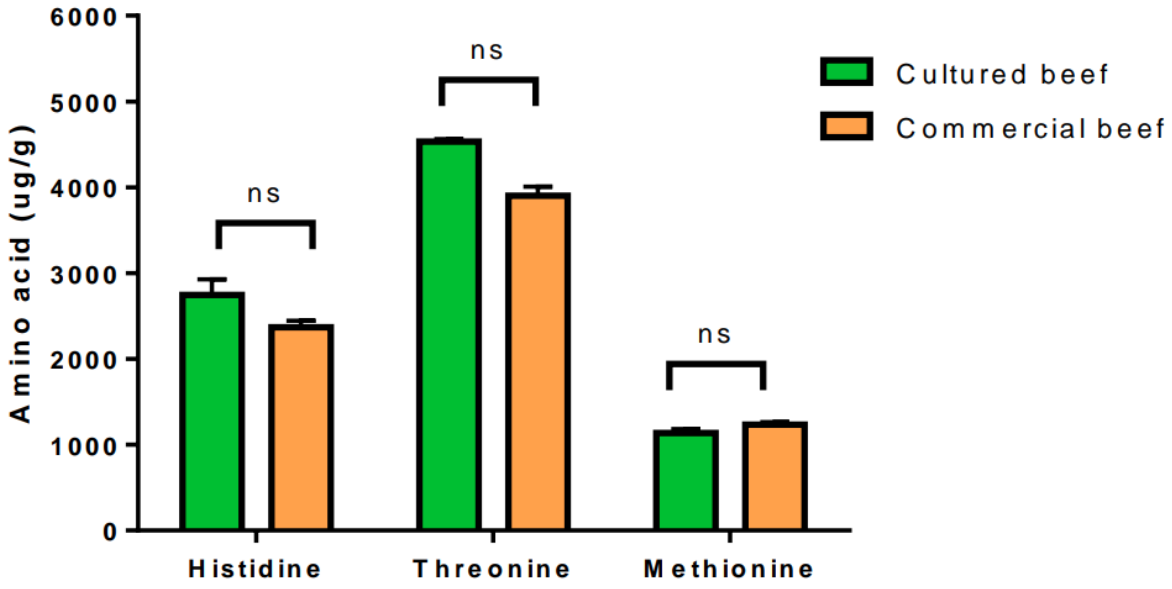
Supplementary Figure 3: 3D suspension culture of bESC (left). bESC-spheroids cultured in a glass spinner flask with NBFR+ medium and 1% F68. Bright field images of spheroids after 8 days of suspension culture and their live/dead rate (right). Scale bars 100 μm.

Supplementary Table 3: The formulation for the production of textured protein scaffolds through the mechanical elongation method.

Wheat gluten (g)	Soy protein isolate (g)	Water (mL)
55	32.8	

Supplementary Table 4: Formulation for scaffold used for cultivated meat.

Scaffold from table S1 (g)	Wheat gluten (g)	Water (mL)	Corn starch (g)	Beet root (g)
13	8	20	6	8



Supplementary Figure 4: The amino acid analysis was conducted using the ultra-high-performance liquid chromatography (UHPLC QE，Thermo，USA) coupled with a high-resolution orbitrap mass spectrometer. The samples underwent extraction at room temperature for 1 hour in the presence of 0.5 mL of 0.1 M hydrochloric acid (HCl), followed by centrifugation at 12,000 rpm for 10 minutes, with subsequent collection of the supernatant. Next, 10 µl of the diluted supernatant was subjected to ultra-high- performance liquid chromatography (UPLC) analysis. The chromatographic column was a Waters BEH C18 (50 x 2.1 mm, 1.7 µm), maintained at 55°C. The injection volume was set at 1 µl, and the flow rate was maintained at 0.5 mL/min. Mobile phases comprised ultrapure water (phase A) and acetonitrile (phase B) containing 0.1% formic acid. The elution gradient proceeded as follows: 0 min 95% A, 5.5 min 90% A, 7.5 min 75% A, 8 min 40% A, 8.5 min 95% A, and 13 min 95% A. P values are labelled: ns (no significance).