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(71) Applicant: Hexie Technology Co., Ltd.
Address: Unit 1, Rm 502, Bldg 6, Yard 88, Kechuang 6th St, BDA, Beijing 102600, China
(72) Inventors: Guihua YANG, Jinjun ZHAO
(74) Agent: Beijing Weizheng Patent Agent Co., Ltd. (11508) / Patent Attorney: Yongjie LU
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A FUNCTIONAL CULTURE MEDIUM FOR DIRECTIONALLY ENHANCING CARTILAGE INJURY REPAIR ABILITY OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS AND APPLICATION THEREOF

ABSTRACT
The present application discloses a functional culture medium for directionally enhancing the cartilage injury repair capacity of human umbilical cord mesenchymal stem cells (hUCMSCs) and applications thereof. On one hand, the present application provides a functional culture medium comprising a basal culture medium and inducing factors. The basal medium includes IMDM medium, insulin, dexamethasone, vitamins, lecithin, human low-density lipoprotein (hLDL), serum replacement, and antioxidants; the inducing factors include TNF-α, IL-1, and IL-6. On the other hand, the present application provides hUCMSCs induced and cultured using the aforementioned medium and their application in products for cartilage injury repair. The present application promotes mesenchymal stem cells to effectively repair necrotic cartilage tissue, suppress inflammatory responses, and promote the survival of nascent chondrocytes.

CLAIMS

1. A functional culture medium for directionally enhancing the cartilage injury repair capacity of human umbilical cord mesenchymal stem cells, characterized in that said medium comprises a basal culture medium and inducing factors, wherein said basal culture medium comprises IMDM culture medium, insulin, dexamethasone, vitamins, lecithin, human low-density lipoprotein, serum replacement, and antioxidants; and said inducing factors comprise TNF-α, IL-1, and IL-6.
2. The functional culture medium according to claim 1, characterized in that the concentration of said insulin in the culture medium is controlled at 10–20 mg/L; and the concentration of said dexamethasone in the culture medium is controlled at 0.5–2 mg/L.
3. The functional culture medium according to claim 1, characterized in that said vitamins comprise a multivitamin mixture including vitamin A, vitamin C, and vitamin E; wherein the concentration of vitamin A in the medium is controlled at 5–10 mg/L, the concentration of vitamin C in the medium is controlled at 10–15 mg/L, and the concentration of vitamin E in the medium is controlled at 5–10 mg/L.
4. The functional culture medium according to claim 1, characterized in that the concentrations of lecithin and human low-density lipoprotein in the medium are controlled at 20–25 mg/L and 10–20 mg/L, respectively; and the concentration of serum replacement in the medium is controlled at 200–400 mg/L.
5. The functional culture medium according to claim 1, characterized in that said antioxidant is reduced glutathione, and the concentration of reduced glutathione in the medium is controlled at 2–4 mg/L.
6. The functional culture medium according to claim 1, characterized in that in said inducing factors, the concentration of TNF-α in the medium is controlled at 5–20 μg/L.

7. The functional culture medium according to claim 1, characterized in that the concentration of IL-1 in the medium is controlled at 5–15 μg/L.
8. The functional culture medium according to claim 1, characterized in that the concentration of IL-6 in the medium is controlled at 10–25 μg/L.
9. Human umbilical cord mesenchymal stem cells prepared by induction culture using the functional culture medium according to any one of claims 1 to 8.
10. Application of the human umbilical cord mesenchymal stem cells according to claim 9 in the field of cartilage injury repair products.

DESCRIPTION

A FUNCTIONAL CULTURE MEDIUM FOR DIRECTIONALLY ENHANCING CARTILAGE INJURY REPAIR ABILITY OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS AND APPLICATION THEREOF

TECHNICAL FIELD

[0001] The present application relates to the field of biomedicine, particularly concerning a functional culture medium for directionally enhancing the cartilage injury repair capacity of human umbilical cord mesenchymal stem cells (hUCMSCs) and applications thereof.

BACKGROUND ART

[0002] With the global aging population, the incidence of osteoarthritis (OA) increases annually. Effective prevention and treatment of OA have become increasingly important medical issues.

[0003] Currently, no conventional or pharmacological therapy has shown clear efficacy in halting disease progression. Injectable molecular compounds, such as corticosteroids, may only alleviate local pain. Surgical intervention can be beneficial when severe deformities exist; however, its efficacy is limited to late-stage OA and does not represent a long-term solution. Autologous Chondrocyte Implantation (ACI) is an option for treating OA, but defects in cost and potential donor site morbidity limit its widespread clinical standard use. Drugs developed to block cartilage degradation only relieve symptoms without curing OA.

[0004] The most widely used clinical treatments follow a traditional pyramidal stepped-care model: conservative treatment in early OA to delay progression, intra-articular drug injections in mid-stage to relieve pain, and surgical joint replacement in end-stage OA. This traditional model is costly, difficult to cure, and results in poor quality of life and significant suffering for patients.

[0005] Mesenchymal stem cells (MSCs) possess plastic adherence under standard culture conditions and the potential to differentiate into osteoblasts, adipocytes, and chondrocytes in vitro. Owing to their multidirectional differentiation potential, immunomodulatory capacity, and self-renewal ability, MSCs have received extensive attention in regenerative medicine. However, despite demonstrated safety in OA treatment, limited efficacy has hindered widespread clinical adoption of standard MSC therapies.

SUMMARY OF THE INVENTION

[0006] To address the aforementioned technical challenges and develop a culture medium that enhances MSC therapeutic efficacy for OA—enabling MSCs to effectively repair necrotic cartilage, suppress inflammation, and promote nascent chondrocyte survival—the present application provides a functional culture medium and applications thereof.

[0007] On one hand, the present application provides a functional culture medium comprising a basal culture medium and inducing factors. The basal culture medium comprises IMDM medium, insulin, dexamethasone, vitamins, lecithin, human low-density lipoprotein (hLDL), serum replacement, and antioxidants; the inducing factors comprise TNF-α, IL-1, and IL-6.

[0008] By adopting this technical scheme, the specific ratio of basal medium components (vitamins, lecithin, hLDL, serum replacement, and antioxidants) effectively promotes hUCMSC growth. The addition of inducing factors (TNF-α, IL-1, and IL-6) promotes the in situ transformation of hUCMSCs into chondrocytes within the joint cavity while directionally boosting their cartilage repair capability. The combination enables hUCMSCs to proliferate and grow in an inflammatory environment, preventing cell apoptosis induced by inflammatory factors.

[0009]-[0016] Specific component concentration ranges in the medium include: insulin (10–20 mg/L), dexamethasone (0.5–2 mg/L), vitamin A (5–10 mg/L), vitamin C (10–15 mg/L), vitamin E (5–10 mg/L), lecithin (20–25 mg/L), hLDL (10–20 mg/L), serum replacement (200–400 mg/L), reduced glutathione (2–4 mg/L), TNF-α (5–20 μg/L), IL-1 (5–15 μg/L), and IL-6 (10–25 μg/L).

[0017] On another hand, the present application provides hUCMSCs prepared by induction culture using the aforementioned functional culture medium, utilizing the following steps:

- S1. Seeding autologous isolated hUCMSCs into culture vessels at a density ≤ 20%, adherent culturing for 24 hours;
- S2. Transferring cells to induction culture vessels, adding in vitro induction medium containing arthritic inflammatory factors at 400–500 mL per 1×10^{9}–10^{10} cells;
- S3. Transferring vessels to a hypoxic incubator, culturing at room temperature for 21–28 days, isolating and purifying inflammatory factor-induced hUCMSCs.

[0020]-[0023] The present application delivers significant beneficial effects: directionally enhancing MSC repair capacity for necrotic cartilage, boosting chondrocyte survival, reducing overall treatment costs, and providing effective early-stage OA therapy.

DETAILED DESCRIPTION

[0024]-[0028] Traditional MSC intra-articular injection therapies face massive cell death due to local inflammatory microenvironments. By introducing inflammatory factors during in vitro induction, the present application pre-conditions MSCs, granting them inflammatory adaptability and inducing differentiation into osteoblasts and chondrocytes prior to transplantation.

EXAMPLES 1–14 FORMULATION RATIOS

Table 1: Basal Medium Component Ratios (Examples 1–14)

Example	Insulin (mg/L)	Dexamethasone (mg/L)	Vitamins (mg/L)	Lecithin (mg/L)	hLDL (mg/L)	Serum Replace. (mg/L)	Antioxidant (mg/L)
1	10	0.5	20	20	10	400	4
2	12	0.7	21	21	11	380	3
3	14	0.9	23	22	12	360	2
4	16	1.1	25	23	13	340	2.5
5	18	1.3	27	24	14	320	3.5
6	20	1.5	29	25	15	300	2
7	19	1.7	31	25	16	280	3
8	17	1.9	33	24	17	260	4
9	15	2.0	35	23	18	240	2
10	13	1.8	34	22	19	220	3
11	11	0.8	32	21	20	200	4
12	14	1.4	26	22	14	320	2
13	16	1.6	28	24	15	360	3
14	18	1.2	30	23	16	340	2.5

Table 2: Inducing Factor Ratios (Examples 1–14)

Example	TNF-α (μg/L)	IL-1 (μg/L)	IL-6 (μg/L)
1	5	5	20
2	6	6	21
3	7	7	23
4	8	8	25
5	9	9	19
6	10	10	17
7	12	11	15
8	14	12	13
9	16	13	11
10	18	14	10
11	20	15	16
12	12	11	18
13	16	10	22
14	14	8	20

EXPERIMENTAL TESTING & IN VIVO VALIDATION

[0031]-[0035] Flow cytometry confirmed >95% positive expression for surface antigens CD105, CD90, CD73, and CD44 across Examples 1–14. Differentiation testing showed 19%–28% osteoblast proportion and 27%–39% chondrocyte proportion.

Table 3: Cell Differentiation & Surface Antigen Test Results

Example	Cell Count (×10^{10})	Osteoblast %	Chondrocyte %	Antigen Expression
1	13.21 ± 1.69	21%	27%	>95% Positive
2	14.27 ± 1.76	19%	29%	>95% Positive
3	15.07 ± 1.89	23%	28%	>95% Positive
4	15.49 ± 1.57	24%	27%	>95% Positive
5	14.42 ± 1.61	23%	31%	>95% Positive
6	14.37 ± 1.94	22%	32%	>95% Positive
7	13.94 ± 1.73	24%	31%	>95% Positive
8	14.18 ± 1.79	26%	33%	>95% Positive
9	14.02 ± 1.86	25%	32%	>95% Positive
10	13.96 ± 1.84	26%	31%	>95% Positive
11	13.39 ± 1.67	28%	34%	>95% Positive
12	15.62 ± 1.93	27%	36%	>95% Positive
13	15.57 ± 1.71	27%	37%	>95% Positive
14	15.68 ± 1.74	26%	39%	>95% Positive

[0036]-[0041] Osteoarthritis rabbit models (papain-induced) treated with hUCMSCs from Examples 1–14 exhibited significant reduction in joint effusion and prominent cartilage repair compared to steroid controls (triamcinolone acetonide) and blank controls.

Table 4: Animal In Vivo Validation Results

Group	Joint Effusion	Articular Cartilage Repair
Examples 1–2	Minor Effusion	Cartilage Repair Observed
Example 3	Trace Effusion	Cartilage Repair Observed
Examples 4–8	No Effusion	Obvious Cartilage Repair
Examples 9–14	No Effusion	Highly Prominent Cartilage Repair
Steroid Control	Minor Effusion	No Obvious Cartilage Repair
Blank Control	Massive Effusion	Aggravated Cartilage Damage