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Different components of chicken ovalbumin extract promote different cell proliferation

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Abstract

The chicken ovalbumin extracts could promote cell survival and proliferation. In the present study, the different components in chicken ovalbumin extracts were further separated to find the component primarily responsible for promoting cell proliferation. Components of differing molecular weight were separated from chicken ovalbumin extracts by ultrafiltration. Different components were co-cultured with different cells at different final concentrations, and the effects on cell proliferation were subsequently determined by a CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega). Components from chicken ovalbumin extracts less than 3 kD in size can promote 293T cell and 293T-GFP cell proliferation. The components from chicken ovalbumin extract more than 3 kD in size can promote bone marrow or umbilical cord mesenchymal stem cell (MSC) proliferation. The separation of components from chicken ovalbumin less than and more than 3 kD in size that are able to function as active components for the promotion of different cellular proliferation. This discovery may identify a new and convenient additive for cell culture media, promoting cell growth and proliferation.

Key words: Chicken ovalbumin extract, components, cell proliferation, less than and more than 3 kD in size.

Introduction

To date, there are many literatures reporting studies about chicken ovalbumin, no extracts from chicken ovalbumin that can promote cell proliferation have been reported in the literature (1-12). Our present study found that components in chicken ovalbumin extracts less and more than 3kD in size can function to promote different cell proliferation and survival. This discovery may identify a new and convenient additive for cell culture media, promoting cell growth and proliferation.

We isolated chicken ovalbumin, egg-yolk and whole-egg extracts and found that both the ovalbumin and whole-egg extracts could promote cell proliferation (13). Further research found that this function resides mainly in the chicken ovalbumin extracts (13). Thus, chicken ovalbumin extracts are easily obtained and have the ability to promote cell proliferation, but the specific components that mediate this function remain unknown. To address this, we conducted intensive studies on chicken ovalbumin extracts. Using ultrafiltration, chicken ovalbumin extracts were divided into the two components based on molecular weights: > 3 kD and < 3 kD. The different components were then co-cultured with 293T cells and mesenchymal stem cell (MSC) at differing final concentrations, and the effect on cellular proliferation was subsequently determined. The study showed that components of chicken ovalbumin extract with < 3 kD and > 3 kD have different effect on proliferation of different cell lines. This paper would have provided new and important data related to the effect of chicken ovalbumin extracts on different cell proliferation.

Materials and methods

Preparation of Chicken egg extracts

The ovalbumin and egg yolk of a chicken egg were sterilely isolated. An equal volume of lysis buffer (50 mM NaCl, 5 mM $MgCl_2$, 100 mM HEPES pH 8.2,1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonylfluoride (PMSF) and protease inhibitor cocktail) was added to the ovalbumin, and the lysis buffer was added to the egg yolk at a 1:3 yolk to buffer ratio. The solutions were fully mixed and frozened and thawed for three times, the solutions were centrifuged, and the supernatants were stored at 4 °C to produce the chicken ovalbumin and egg yolk extracts. To generate the whole-egg extracts, a separate chicken egg was sterilely isolated, lysed using a 1:2 egg to lysis buffer ratio, fully mixed and frozened and thawed for three times. Then the solution was centrifuged, and the supernatant was stored at 4 °C. The protein concentrations of the extracts were measured using the Bradford method. The extracts were then marked with their protein concentrations and preparation times, aliquoted and stored at -20 °C for later use. To visualize a stable standard for this natural product a minimum of 6 charges were used in our experiments.

Co-culture with 293T cells

The source of 293T cells was purchased from Kunming Cell Bank of Chinese Academy of Sciences. 293T cells were seeded in 6-well plates, 3×10^5 cells each well for four wells, 1ml of complete medium was added. The complete medium is DMEM-F12 medium containing 10% fetal calf serum. And then sequentially 1ml

of complete medium, chicken ovalbumin extract, chicken egg yolk extract, chicken whole egg extract were added in four wells for co-culture, the cell viability was imaged the following day. The survival rate with different extracts was quantified. The experiments have repeated for more than three times and collected the similar results. Through the experiment we have demonstrated chicken ovalbumin extract have roles to promote cell survival. Then a CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega) was used to detect cell growth and proliferation under different culture conditions.

Separation of different chicken ovalbumin extracts components

Chicken ovalbumin extracts were separated using ultrafiltration tubes (molecular weight cut-offs of 3 kD) with a processing capacity of 15 ml. A total of 15 mL of chicken ovalbumin extract was applied to the ultrafiltration tube and centrifuged at 4000 rpm for 30 min; the sample passing through the filter representing components less than 3 kD in size. The sample no passing through the filter represent components more than 3 kD in size.

SDS-PAGE electrophoresis

Chicken ovalbumin extract and greater than 3 kD components were prediluted 20 times, and 50 μL each of the three components of the sample were added 50 μL 2 × sample buffer, boiling water bath for 5 min. 5 μL sample was loaded to the well, electrophoresis reached to the bottom of the gel. The gel was stained for 3 hours, decolored overnight and camera observations.

Detection of cell proliferation

A suspension of different cells was adjusted to a concentration of 1×10^5 cells/mL, and 100 μL PBS per well was added to the outer perimeter wells of a 96-well plate (36 wells in total) to prevent evaporation. The cells we used included 293T, 293T-GFP, bone marrow mesenchymal stem cell from tree shrew (TS-BMSC) and from C57BL mice (C57-BMSC), and umbilical cord mesenchymal stem cell from tree shrew (TS-UC-MSC). The criteria for the selection of the cells were these cell lines were often cultured in our laboratory and through the identification of standard cell lines. The growth of the in vitro cultured cells have been analyzed in terms of cell number, cell transformation and other parameter such as cell state etc., to ensure the isolate did not interfere the growth of the cells. Then, 50 μl cells per well (5×10^3 cells per well) were added to the remaining wells of the plate. Next, 50 μL of extract was added to achieve a final concentration of 50 %. The extract was prediluted with medium to create a 50 % stock extract. Then, 50 μl of the stock extract was added to each well for a final concentration of 25 % extract. As such, extract was added to each well for a final concentration of 50 %, 40 %, 30 %, 25 %, 20 % extract. Each extract was added to 10 wells, and the plate was incubated at 37 °C for 3-4 days. A CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega) was then used to detect cell viability. Briefly, 20 μL of reagent was added to each well followed by incubation at 37 °C for 3 hours and detection at 490 nm using a colorimeter.

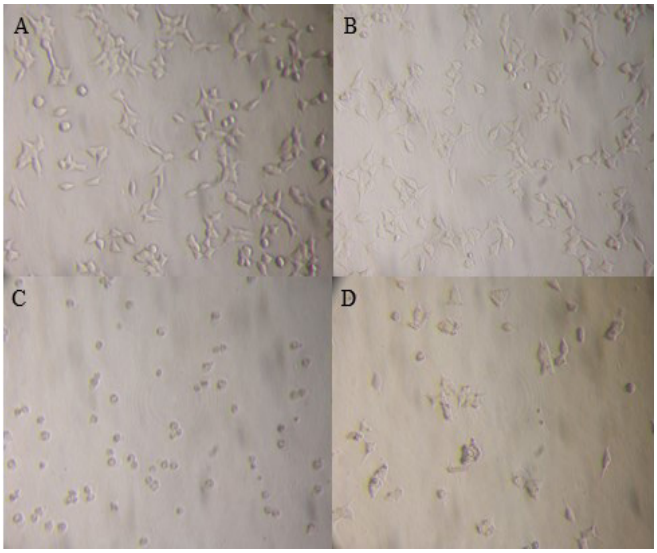

Figure 1. Survival results of cells after co-culture with different extracts. The experiments have repeated for more than three times with the similar results. A: media. B: ovalbumin extracts. C: egg-yolk extracts. D: whole-egg extracts.

Statistical Analysis

The data values are shown as the mean ± SD. Groups were compared using one-way ANOVA by SPSS 17.0 statistical software. Multiple comparisons (LSD) were used after one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Differing roles of three chicken egg extracts in the promotion of cell survival and growth

Chicken ovalbumin extracts most strongly promoted 293T cell survival and growth, followed by the whole-egg extracts. Egg-yolk extracts had the smallest effect on the growth of cells (Figure 1). By the second day after co-culture, all of the cells cultured with ovalbumin extract survived and were adherent, while only a portion of the cells cultured with whole-egg extract survived. The cells cultured with egg-yolk extract had the smallest number of surviving cells. How the ovalbumin extracts function to better promote cell growth is worthy of further study. The survival rate with different extracts was quantified. The cell survival rate with chicken ovalbumin extracts is 100%, with egg-yolk extracts is 5%, and with whole-egg extract is 30%.

SDS-PAGE results of chicken ovalbumin extract

The protein concentrations were measured by the Bradford method, the results were as following:

Extract	Protein concentration (mg/mL)
Chicken ovalbumin extract	24
Greater than 3 kD components	31
Less than 3 kD components	0.14

Chicken ovalbumin extract was separated by ultrafiltration to > 3 kD and < 3 kD components. The electrophoresis results showed that the chicken ovalbumin extracts have three main bands, 90 kD, 40 kD, 12 kD (Figure 2).

SDS-PAGE electrophoresis showed less than 3 kD components have no protein content. The greater than 3 kD components have a protein concentration greater

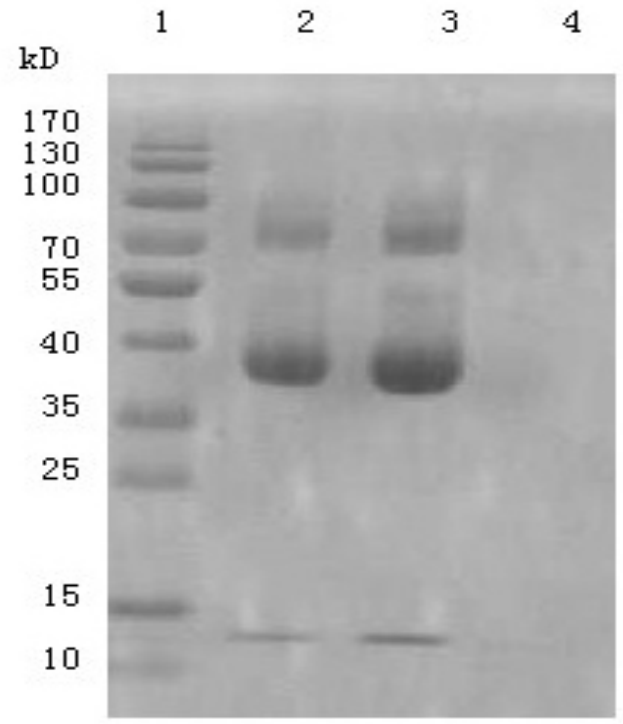

Figure 2. Electrophoresis of the different components of chicken ovalbumin extract. A protein molecular weight standards. 2 chicken ovalbumin extract. 3 greater than 3 kD components in chicken ovalbumin extract. 4 less than 3 kD components in chicken ovalbumin extract.

than chicken ovalbumin extract.

Differing roles of various components of chicken egg extracts in promoting proliferation when co-cultured with 293T cells

As the figure 3 shows, the < 3 kD components of chicken ovalbumin extract (50% final concentration) promotes 293T cell proliferation more strongly than the chicken ovalbumin, egg-yolk or whole-egg extracts but less strongly than media. The chicken ovalbumin extracts promote 293T cell proliferation more strongly than either the egg-yolk or whole-egg extracts. We therefore focused our studies on chicken ovalbumin extracts. As the figure 3E and 3F shows, the < 3 kD components from chicken ovalbumin extracts (final concentration of 25%) promoted 293T cell proliferation more strongly than the commonly used media ($P < 0.05$). When < 3 kD components with final concentration of 25%, the fetal calf serum is only with final concentration of 7.5%. Since the fetal calf serum can promote cell proliferation, at the same time, the commonly used media were with 10% final concentration of the fetal calf serum. When the fetal calf serum with final concentration of 7.5%, < 3 kD components with final concentration of 25%, the promote cell proliferation function is stronger than the media with 10% final concentration of the fetal calf serum. This result identifies the < 3 kD components in chicken ovalbumin extracts as a potential additive for 293T cell culture media to better promote 293T cell proliferation.

Differing roles of various components of chicken egg extracts in promoting proliferation when co-cultured with 293T-GFP cells

As observed from the figure 4C, the < 3 kD components from chicken ovalbumin extract (25% final concentration) promotes 293T-GFP cell proliferation more strongly than the commonly used media. At this time, the fetal calf serum with final concentration of 7.5%, the promote cell proliferation function is stronger than the media with 10% final concentration of the fetal

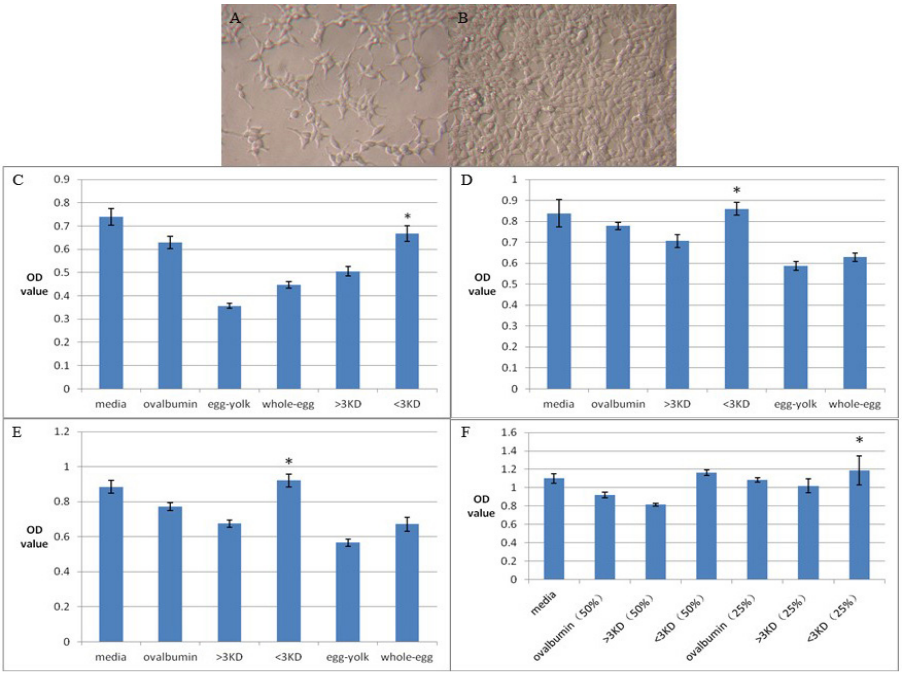

Figure 3. 293T cell morphology and differing effects on proliferation of various components of chicken egg extracts co-cultured with 293T cells. A: Adherent 293T cells. B: 293T cell should be passaged. C: 50 % final concentration of various components co-cultured with 293T cells for two days (n = 10) (* $P < 0.01$ compared with any group). D: 20 % final concentration of various components co-cultured with 293T cells for three days (n = 10) (* $P > 0.05$ compared with the medium group, $P < 0.01$ compared to the other groups). E: 25 % final concentration of various components co-cultured with 293T cells for three days (n = 10) (* $P < 0.05$ compared with the medium group, $P < 0.01$ compared to the other groups.). F:Differing roles of different concentrations of each ovalbumin extract component in promoting proliferation when co-cultured with 293T cells for 3 days (n = 10) (*$P = 0.408$ when < 3 kD (25 %) compared to < 3 kD (50 %), $P < 0.01$ compared to the other group).

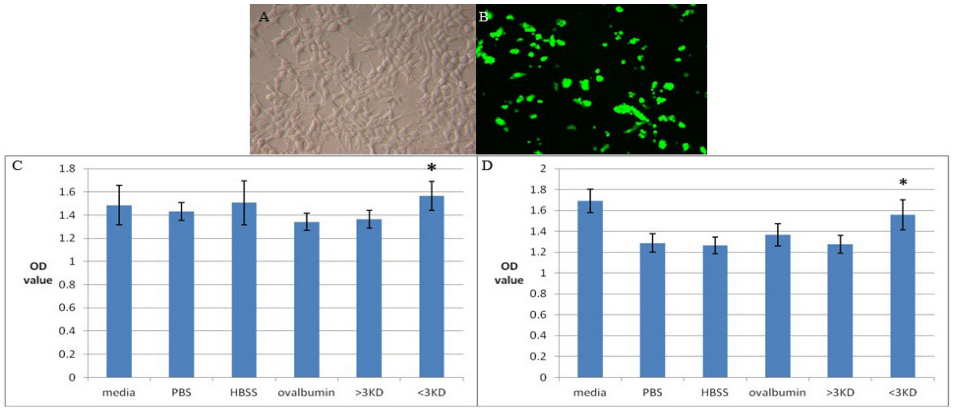

Figure 4. 293T-GFP cell morphology and differing effects on proliferation of various components of chicken egg extracts co-cultured with 293T –GFP cells. A: 293T-GFP cell under light microscope. B: 293T-GFP cell under fluorescence microscope. C: 25 % final concentration of various components co-cultured with 293T-GFP cells for three days (n = 10) (* $P < 0.05$ compared with PBS, ovalbumin and > 3 kD groups, $P > 0.05$ compared with the medium group and HBSS group). D: 50 % final concentration of various components co-cultured with 293T-GFP cells for three days (n = 10) (* $P < 0.01$ compared with any group).

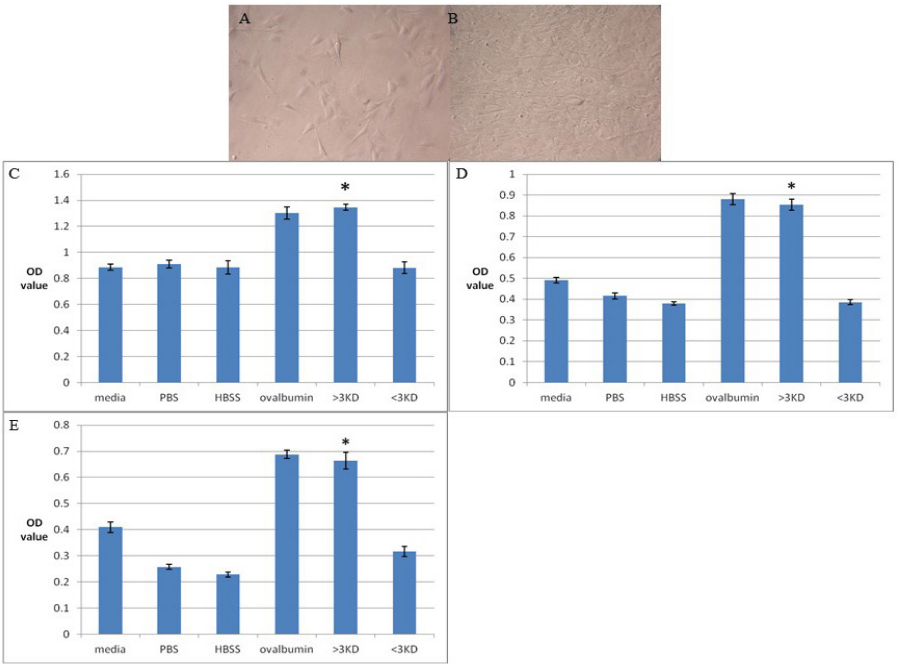

Figure 5. TS-BMSC morphology and different proliferation effects of various components of chicken egg extracts co-cultured with TS-BMSC cells. A: Adherent TS-BMSC. B: TS-BMSC should be passaged. C: 20 % final concentration of various components co-cultured with TS-BMSC cells for four days (n = 10) (* $P < 0.01$ compared with the medium group). D: 25 % final concentration of various components co-cultured with TS-BMSC cells for four days (n = 10) (* $P < 0.01$ compared with the medium group). E: 50 % final concentration of various components co-cultured with TS-BMSC cells for four days (n = 10) (* $P < 0.01$ compared with the medium group).

calf serum. This result identifies the < 3 kD components of chicken ovalbumin extract as a potential additive for 293T-GFP cell culture media to better promote 293T-GFP cell proliferation.

Differing roles of various components of chicken ovalbumin extracts in promoting proliferation when co-cultured with bone marrow mesenchymal stem cell from tree shrew (TS-BMSC)

As observed from the figure 5, the > 3 kD components from chicken ovalbumin extract (20%, 25%, 50% final concentration) and chicken ovalbumin extract promotes TS-BMSC cell proliferation more strongly than the commonly used media ($P < 0.01$). 20%, 25%, 50% final concentration of extract is only with 8%, 7.5%, 5% final concentration of fetal calf serum. The final concentration of fetal calf serum at this time is lower than media with 10% fetal calf serum. But the promote cell proliferation function is stronger than media. This result identifies the > 3 kD components of chicken ovalbumin extract and chicken ovalbumin extract as potential additives for TS-BMSC cell culture media to better promote TS-BMSC cell proliferation.

Differing roles of various components of chicken ovalbumin extracts in promoting proliferation when co-cultured with bone marrow mesenchymal stem cell from C57BL mice (C57-BMSC)

As observed from the figure 6 (except Figure 6D), the > 3 kD components from chicken ovalbumin extract (25%, 40%, 50% final concentration) and chicken ovalbumin extract promotes C57-BMSC cell proliferation more strongly than the commonly used media ($P < 0.01$). 25%, 40%, 50% final concentration of extract is only with 7.5%, 6%, 5% final concentration of fetal calf serum. The final concentration of fetal calf serum at this time is lower than media with 10% fetal calf serum. But the promote cell proliferation function is stronger than

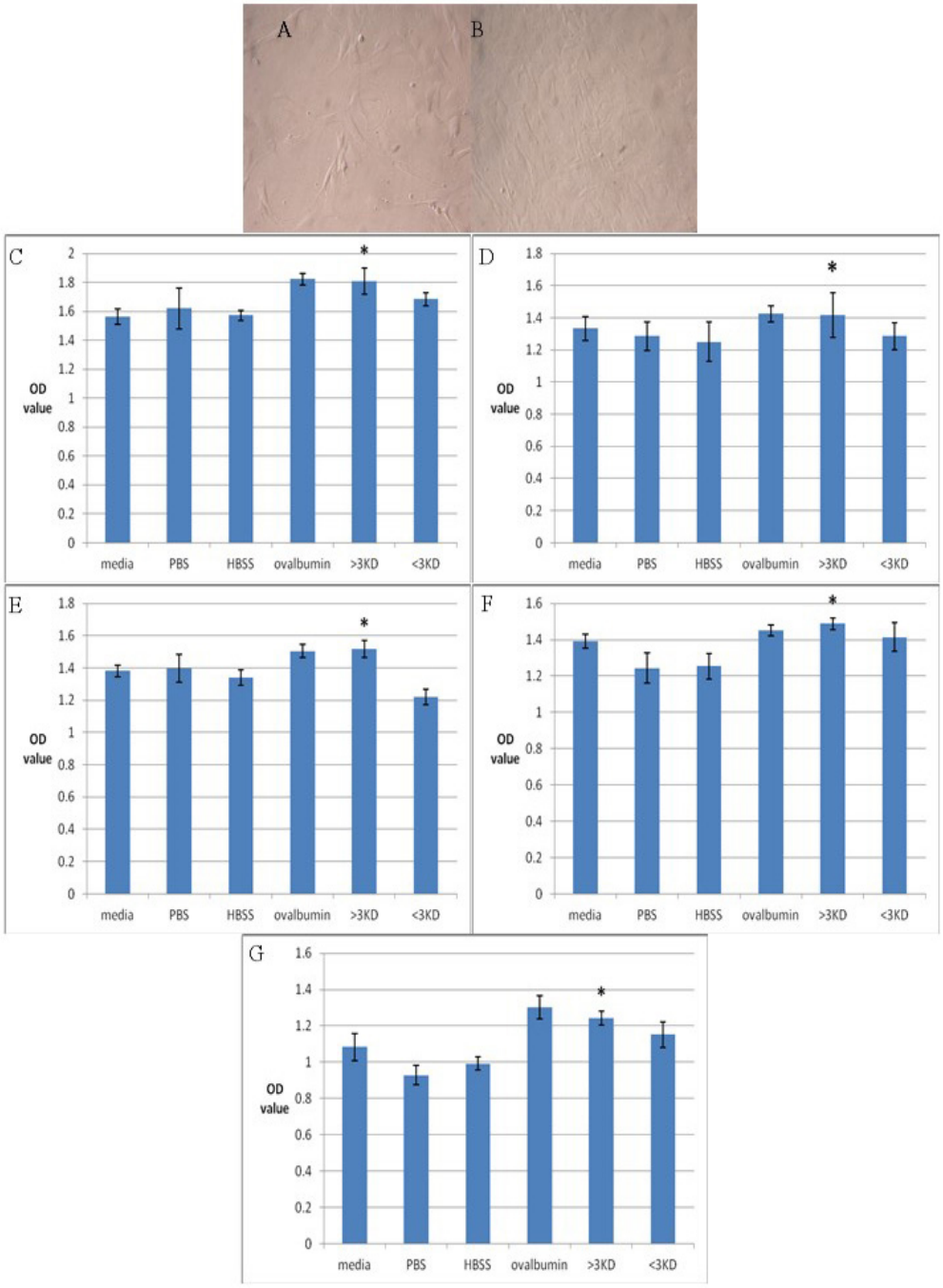

Figure 6. C57-BMSC morphology and different proliferation effects of various components of chicken egg extracts co-cultured with C57-BMSC cells. A: Adherent C57-BMSC. B: C57-BMSC should be passaged. C: 25 % final concentration of various components co-cultured with C57-BMSC cells for two days (n = 10) (* $P < 0.01$ compared with the medium group). D: 20 % final concentration of various components co-cultured with C57-BMSC cells for three days (n = 10) (* $P > 0.05$ compared with the medium group and ovalbumin group, $P < 0.01$ compared with the other group). E. 25 % final concentration of various components co-cultured with C57-BMSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group). F: 40 % final concentration of various components co-cultured with C57-BMSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group). G: 50 % final concentration of various components co-cultured with C57-BMSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group).

media. This result identifies the > 3 kD components of chicken ovalbumin extract and chicken ovalbumin extract as potential additives for C57-BMSC cell culture media to better promote C57-BMSC cell proliferation.

Differing roles of various components of chicken ovalbumin extracts in promoting proliferation when co-cultured with umbilical cord mesenchymal stem cell from tree shrew (TS-UC-MSC)

As observed from the figure 7(except Figure 7I), the > 3 kD components from chicken ovalbumin extract (25%, 30%, 40%, 50% final concentration) and chicken ovalbumin extract promotes TS-UC-MSC cell proliferation more strongly than the commonly used media ($P < 0.01$). 25%, 30%, 40%, 50% final concentration of extract is only with 7.5%, 7%, 6%, 5% final concentration of fetal calf serum. The final concentration of fetal calf serum at this time is lower than media with 10% fetal calf serum. But the promote cell proliferation function is stronger than media. This result identifies the > 3 kD components of chicken ovalbumin extract and chicken ovalbumin extract as potential additives for TS-UC-MSC cell culture media to better promote TS-UC-MSC cell proliferation.

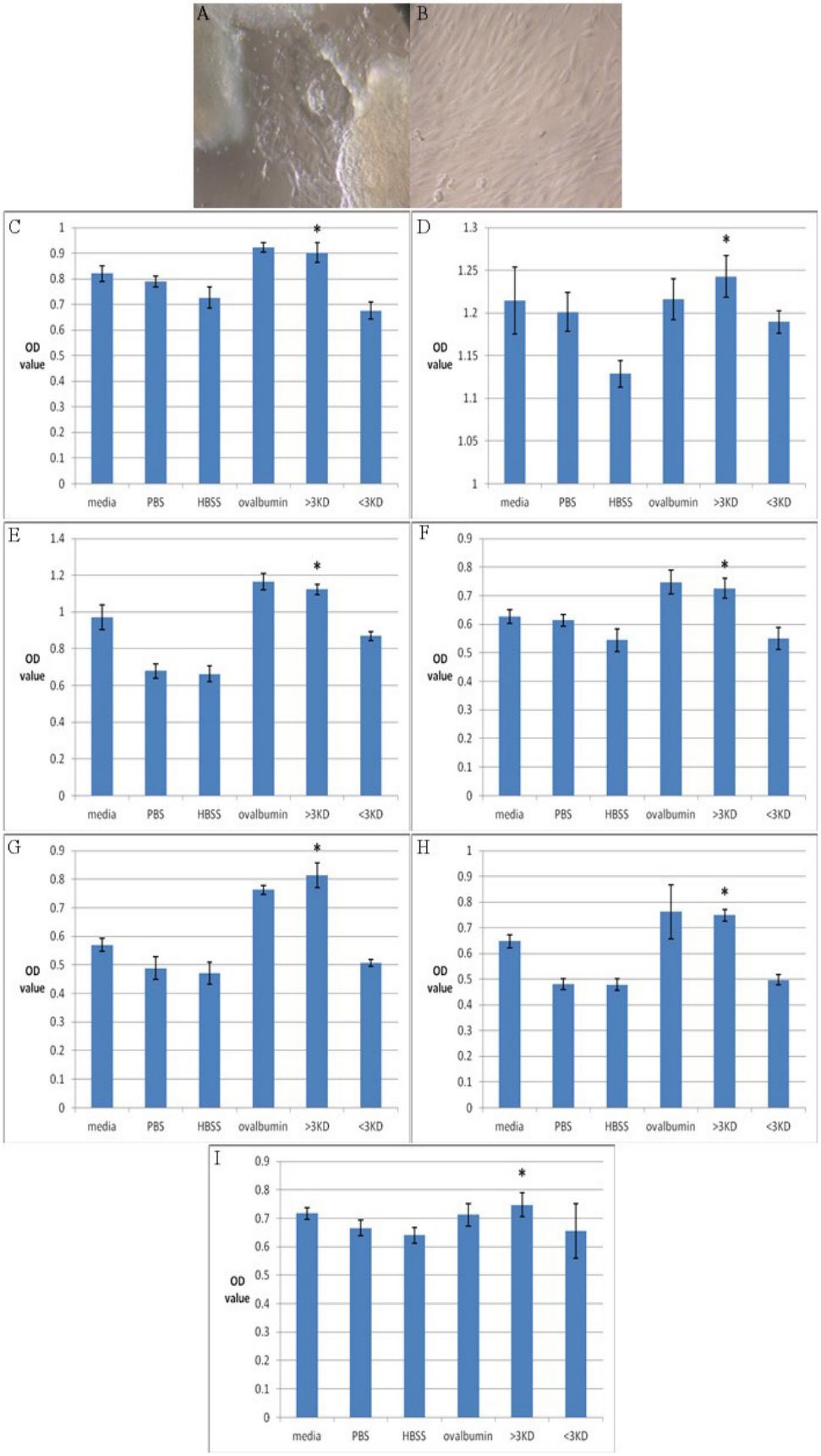

Figure 7. TS-UC-MSC morphology and different proliferation effects of various components of chicken egg extracts co-cultured with TS-UC-MSC cells. A: Adherent TS-UC-MSC. B: TS-UC-MSC should be passaged. C: 25 % final concentration of various components co-cultured with TS-UC-MSC cells for two days (n = 10) (* $P < 0.01$ compared with the medium group). D: 30 % final concentration of various components co-cultured with TS-UC-MSC cells for two days (n = 10) (* $P < 0.01$ compared with the medium group). E: 50 % final concentration of various components co-cultured with TS-UC-MSC cells for two days (n = 10) (* $P < 0.01$ compared with the medium group). F: 25 % final concentration of various components co-cultured with TS-UC-MSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group). G: 30 % final concentration of various components co-cultured with TS-UC-MSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group). H: 40 % final concentration of various components co-cultured with TS-UC-MSC cells for three days (n = 10) (* $P < 0.01$ compared with the medium group). I: 50 % final concentration of various components co-cultured with TS-UC-MSC cells for three days (n = 10) (*$P > 0.05$ compared with the medium group and ovalbumin group, $P < 0.01$ compared with the other group).

Discussion

The chicken egg yolk is the largest component of the egg, and the yolk membrane functions similarly as a cell membrane (8, 9). The ovalbumin and eggshell, which have roles in nutrition and protection, are formed from oviduct secretions. We prepared chicken ovalbumin, egg-yolk, and whole-egg extracts, used them to treat 293T, 293T-GFP, TS-BMSC and C57-BMSC, and TS-UC-MSC, and observed the effects on cell survival and differentiation. The results showed that chicken ovalbumin extract contained substances that promoted 293T, 293T-GFP, TS-BMSC and C57-BMSC, and TS-UC-MSC survival and proliferation. Now we don't know the nature of the key factors which have a proliferation-enhancing function. In the future, this could be conducted by treating the isolates with different PH and temperature. The criteria for the selection of the cells were these cell lines were often cultured in our laboratory and through the identification of standard cell lines. The effects were significantly different than those observed with the control. We assume that these substances are proteins or small molecules in the chicken egg. In our follow-up studies, we found that chicken ovalbumin extracts play an important role in promoting various cell survivals.

In this study we found that chicken ovalbumin extracts could promote the growth and proliferation of cells, although we did not identify the specific factors mediating this effect. In this experiment, we separated the chicken ovalbumin extracts into different components of > 3 kD and < 3 kD. These different components were co-cultured with different cells at differing final concentrations with different days, and the results showed that the < 3 kD components is capable of promoting 293T and 293T-GFP cell proliferation, while > 3 kD components and chicken ovalbumin extract are capable of promoting TS-BMSC, C57-BMSC and TS-UC-MSC cell proliferation. When the final concentration of the < 3 kD components was 25%, a stronger effect on 293T and 293T-GFP proliferation was observed than with media only. This result suggests that the < 3 kD components of chicken ovalbumin extracts are a potential additive for 293T and 293T-GFP cell culture media to better promote cell proliferation. When the different final concentration of the > 3 kD components and chicken ovalbumin extract were applied with TS-BMSC, C57-BMSC and TS-UC-MSC cell, these cells proliferation were observed than with media only. This result suggests that the > 3 kD components of chicken ovalbumin extracts are a potential additive for MSC cell culture media to better promote MSC cell proliferation, < 3 kD components of chicken ovalbumin extracts are a potential additive for somatic cells line, such as 293T and 293T-GFP to better promote somatic cell proliferation.

In our cell proliferation assays, we added the extracts to different final concentrations, and cells were subsequently cultured for 2, 3, or 4, 5 days. Due to the cumbersome steps associated with traditional MTT assays, which introduce many factors that could affect the test, we determined MTT assays to be unsuitable for the present study. We therefore used an imported cell viability detection kit that simply requires the addition of 20 μL reagent per well, incubation at 37 °C for 3 hours, and detection at 490 nm on a colorimeter. This simplified the procedure, removing the need to siphon off the supernatant or dissolve the precipitate. After co-culture, the data were read directly on a colorimeter, making the results more accurate and more reproducible. Due to an edge effect in the 96-well plates, the wells located on the edge of the plate could produce inaccurate results due to evaporation. We excluded the 36 wells located the edges and instead added 100 μL PBS to each of these wells to prevent these edge effects and ensure accurate results. Each concentration of each extract was added to 10 wells, and we determined the mean ± standard deviation for each set to make the results more comparable.

Our results indicate that components that are < 3 kD in chicken ovalbumin extracts at a final concentration of 25 % more strongly promote 293T and 293T-GFP cell growth than the culture media only. The > 3 kD components and chicken ovalbumin extracts are a potential additive for TS-BMSC, C57-BMSC and TS-UC-MSC cell culture media to better promote cell proliferation. We can now consider isolating the components that are < 3 kD in chicken ovalbumin extracts for use as 293T and 293T-GFP cell media additives to promote cell growth and proliferation, and isolating the > 3 kD fractions and chicken ovalbumin extracts for use as TS-BMSC, C57-BMSC and TS-UC-MSC cell media additives to promote cell growth and proliferation.

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