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Soy hydrolysate mimic autocrine growth factors effect of conditioned media to promote single CHO-K1 cell proliferation

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ABSTRACT

The increasingly competitive biopharmaceutical industry requires companies to focus on rapid and low-cost cell line development. Single-cell cloning (SCC) is a critical and high-value process for cell line development, and typically problematic because single cell proliferates slowly when cultivated at low cell densities. Conditioned media (CM) provide autocrine growth factors to facilitate single cell proliferation, thus improve SCC efficiency. However, conditioned media (CM) are not a feasible solution for industrial cell line development due to variation and cross-contamination concerns. Here, we have found an improvement in the SCC efficiency similar to CM when soy hydrolysate was supplemented in SCC media. Therefore, we concluded that hydrolysate can mimic the autocrine growth factor(s) effect to improve cloning efficiency observed in CM.

1. Introduction

The commercial success of monoclonal antibodies (Mabs) has made biological therapeutics attractive to pharmaceutical companies (Ledford, 2010). The priority of biopharmaceutical companies is to acquire and develop cell culture technology platforms that enable them to manufacture biologics quickly, consistently and economically. The increasingly competitive biopharmaceutical industry requires companies to focus on rapid and low-cost cell line development (Gonçalves et al., 2016; Hou et al., 2014; Hurst et al., 2014; Le et al., 2015; Orlova et al., 2012) Single cell cloning (SCC) is a critical and high value process for cell line development (Yuan et al., 2017). However, single cell proliferates slowly, or even do not survive when cultivated at low cell densities (Lee et al., 1992; Ozturk and Palsson, 1990). The poor efficiencies of single-cell proliferation lead to long development time for SCC during cell line development (Lim et al., 2013). Conditioned media which is typically derived from exponentially growing cell cultures provide autocrine growth factors to facilitate single cell proliferation, thus improve SCC efficiency (Lim et al., 2013). However, conditioned media (CM) are not a feasible solution for industrial cell line development due to variation and cross contamination concern (Dhulipala et al., 2011; Lim et al., 2013). The autocrine growth factors in CM were previously identified and added to SCC media to promote single cell proliferation (Lim et al., 2013). However, the industrial production of these SCC media is difficult since the supply of these autocrine growth factors is expensive and unstable. Therefore, an alternative raw material that can mimic the effect of autocrine growth factors is needed to build an SCC media with consistent supply. Hydrolysate has long been used as a protein replacement in cell culture media (Farges-Haddani et al., 2006; Girón-Calle et al., 2008). Therefore, we have investigated the effect of the hydrolysate to improve SCC efficiency in our current study.

2. Methods & Materials

2.1. Cell culture

Suspension culture the CHO-K1 cell line was purchased from Public Health England. Cells are directly suspended in QUACELL CD02 media. Adaptation was done by passaging the cells to a viable cell density of 0.5×10^6 cells/ml, when the viable cell density (VCD) reached 3×10^6.

2.2. Media

Condition media (Quacell) were also collected as previously described (Lim et al., 2013). SCC media were previously developed in house using DMEM media (Quacell) with amino acid adjustment as

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previously reported with modifications (Lu et al., 2013). Other SCC media were purchased from Irvine Scientific. Cotton, Pea and Yeast Hydrolysate samples were obtained from Kerry for testing.

2.3. Single-cell cloning experiments

SCC Experiments were performed as previously described with modifications (Dhulipala et al., 2011; Lim et al., 2013). Briefly, 100 uL of different compositions of SCC medium were added to each well in a 96-well plate except for A1 well. 200 uL of cell suspension were added to A1 well. Then quickly 100 uL of cell suspension from A1 was transferred into B1. This process was repeated to dilute the cells in the first column from A1 to the H1 well. After this dilution, 100 uL of culture medium were added into each well in the first column to make the final volume to 200 u L. 100 uL of cell suspension from the first column were transferred into the second column. The process was repeated to dilute column by column until the twelfth column to make uo the volume to 200 u L. The 96 well plates were incubated at 37 °C with 5% CO^2 in a humidified incubator. The plates were scanned by Cell-Metric Imager (Solentium) to identify single cell clones at 0 h, 24 h, 48 h, 96 h. Proliferated single cells were identified by Cell-Metric Imager at 96 h.

3. Result and Discussion

Single CHO-K1 cell proliferation is a slow and random process during SCC (Lee et al., 1992; Ozturk and Palsson, 1990). Therefore, some single CHO-K1 cells stop proliferation after the first or second cell division. Fig. 1a and Fig. 1b shows a single CHO-K1 cell ceased to proliferate after first or second cell division respectively. Therefore, single CHO-K1 cells are considered proliferated only after the fourth cell division in our current study (Fig. 1c). In our current study, SCC efficiency is measured by the percentage of proliferated single cells of all single cells in a 96 well (Eq. 1).

$$\text{SCC Efficiency} = \text{No. of Proliferated Cells/No. of Total Single Cells} \quad (1)$$

Our in-house SCC media has shown low SCC efficiency (6%) as previously reported (Lim et al., 2013), indicating that only 6% of single CHO-K1 cells proliferated beyond the fourth generation in our in-house SCC media. However, CM media can significantly increase SCC efficiency to 74% (Fig. 1d). This result indicates that the effect of autocrine growth factors in CM can promote single cell proliferation and improve SCC efficiency as previously reported (Lim et al., 2013).

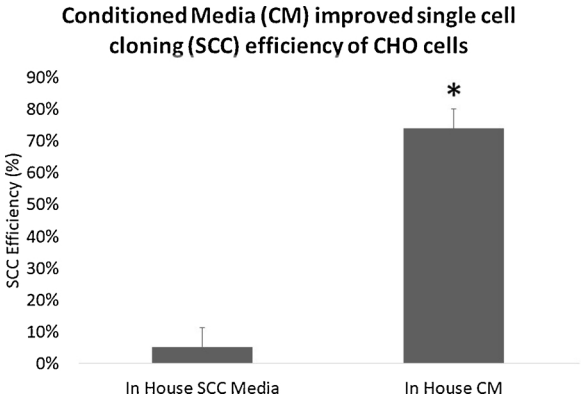

Fig. 2. shows SCC efficiency (%) was significantly increased in conditioned media (CM). Error bars indicate S.D. calculated from six replicates. $^{*}p < 0.005$.

To identify an alternative raw material that can mimic the effect of autocrine growth factors in CM, we have investigated the effect of different types of hydrolysate on cloning efficiency (Fig. 2). While cotton and pea hydrolysate have increased the cloning efficiency to 22% and 16% respectively in our in-house SCC media, it did not show any statistical significance. Whereas, supplementation of soy hydrolysate showed a significant statistical improvement of cloning efficiency to 39% (Fig. 3). Further, to improve the SCC efficiency to a similar level of CM, the concentration of the soy hydrolysate was optimized (Fig. 4). We found that 1.5X of the hydrolysate concentration was able to improve the clone efficiency to 66%, which is statistically significant when compared to the SCC efficiency of our in-house CM (Fig. 4).

To evaluate whether soy hydrolysate can improve cloning efficiency in other SCC media formulation, cloning efficiency of a commercial SCC media were compared to that of the same commercial SCC media with soy hydrolysate supplementation. Even though there was an improvement in clone efficiency, the improvement was comparatively less obvious (39%) (Fig. 5). We speculated that the less obvious improvement might be due to the sensitivity among different media formulations to the autocrine growth factors effect of the soy hydrolysate. Nevertheless, we found that soy hydrolysate can mimic the autocrine growth factor effect to improve cloning efficiency observed in CM in our current study.

Moreover, we demonstrated that hydrolysate can improve the cloning efficiency similar to that of the autocrine growth factors in CM, observed in the previous study (Lim et al., 2013). The higher cloning

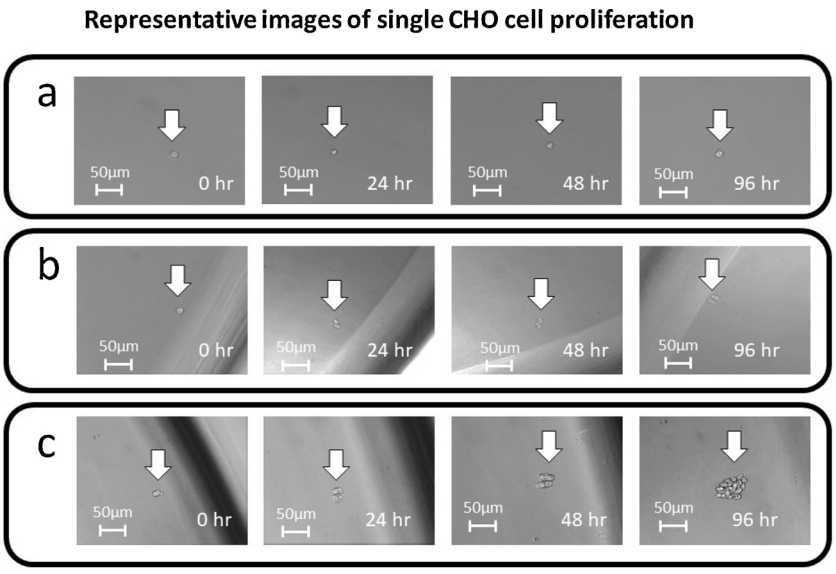

Fig. 1. shows a representative single CHO-K1 cell proliferation during SCC. (a) a single CHO-K1 cell was not divided after plating. (b) a single CHO-K1 cell stop proliferation after one division (24 h). (c) a single CHO-K1 cell divided continuously, and division more than four generations were considered proliferated cells. Arrows indicate the proliferation of from a single cell.

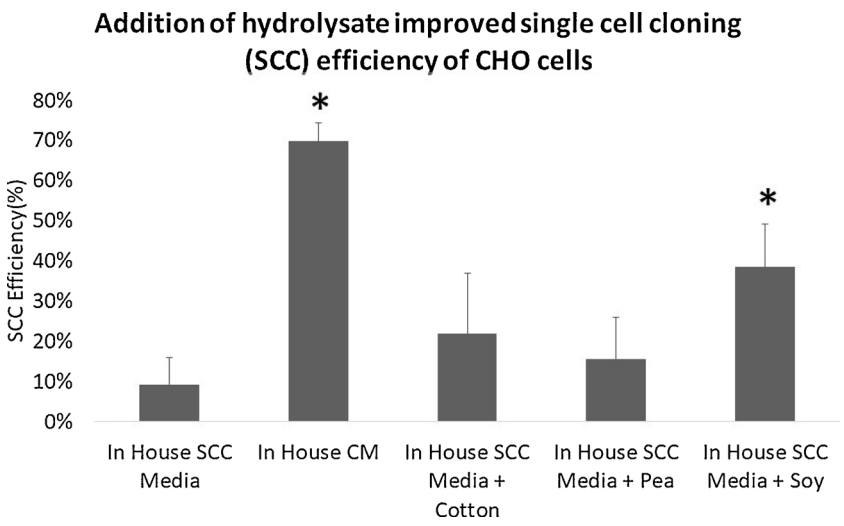

Fig. 3. show SCC efficiency (%) was significantly increased inhouse SCC media with soy hydrolysate supplementation when compared to that without soy hydrolysate supplementation. No statistical significance was observed with cotton and pea hydrolysate wwas supplemented. Error bars indicate S.E.M. calculated from six replicates. $^{*}p < 0.005$.

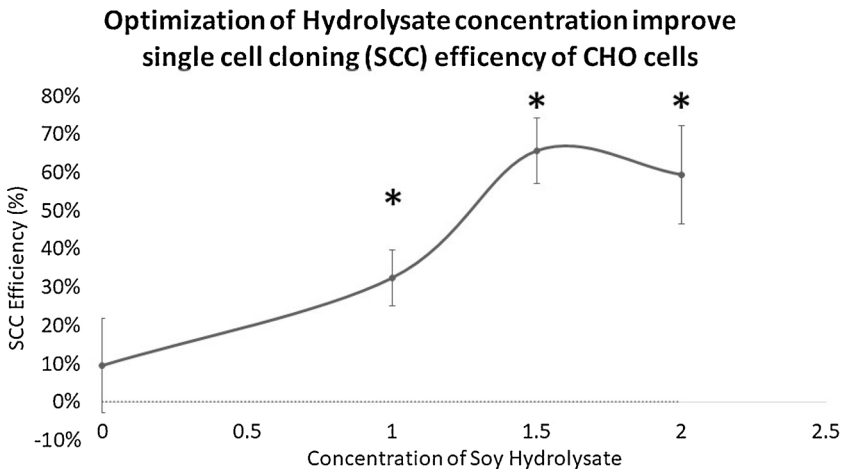

Fig. 4. show SCC efficiency (%) was significantly increased in our in-house SCC media with different concentration of soy hydrolysate supplementation when compared to our in-house SCC media without soy hydrolysate supplementation. The concentration of soy hydrolysate was increased to 1.5x and 2x based on the concentration of soy hydrolysate in Fig. 3 (1x). Error bars indicate S.E.M. calculated from six replicates. $^{*}p < 0.005$.

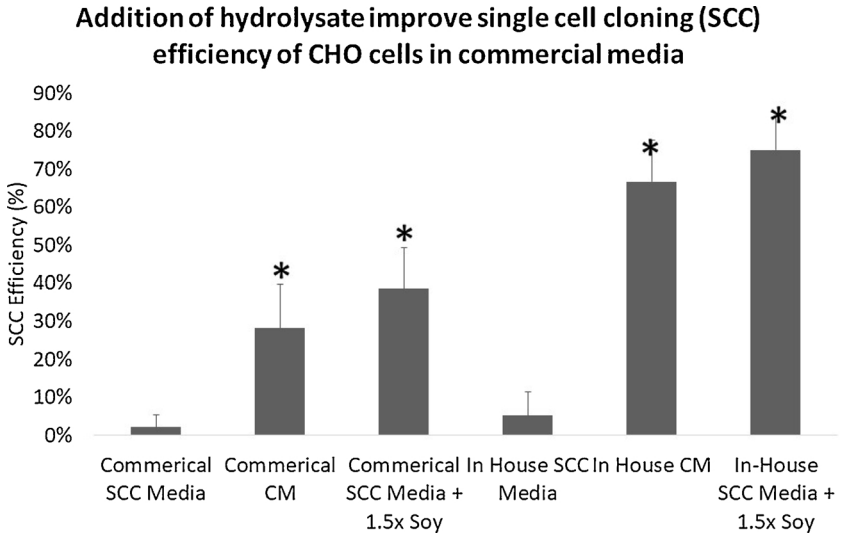

Fig. 5. show SCC efficiency (%) was significantly increased in a commercial SCC media with soy hydrolysate supplementation when compared to that without soy hydrolysate supplementation. Similar observation was observed in our in-house SCC media. Error bars indicate S.E.M. calculated from six replicates. $^{*}p < 0.005$.

efficiencies were speculated due to the complementary effects of various signalling pathways and growth mechanisms (Lim et al., 2013). It has shown that hydrolysate can be used as a protein replacement in cell culture media (Farges-Haddani et al., 2006; Girón-Calle et al., 2008). Therefore, it is perusable that the peptides within the soy hydrolysate activate the autocrine singling pathway to improve cloning efficiency (Lim et al., 2013; Lu et al., 2013; Pak et al., 1996). The identification of specific peptides and canonical pathways relevant to improve cloning efficiency are in progress.

Conflict of interest

Authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors

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