METHODS AND SYSTEMS FOR THE SCALABLE GROWTH AND MAINTENANCE OF STEM CELLS USING OPTOGENETICS IN SUSPENSION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/224,178, filed July 21, 2021, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Cell production is currently required at a scale not seen before, with demand set to increase dramatically. Of particular interest is the production of stem cells; however, control of the processes that maintain stemness is complex. Stem cells generally require one or more protein factors (e.g., growth factors) present in the culture media to promote growth and/or maintenance (of stemness). Successfully culturing these cells requires tightly-controlled combinations and proportions of growth factors. However, these growth factors are extremely expensive to produce, making large-scale growth and maintenance of stem cells prohibitively expensive. In addition, two-dimensional culture of stem cells (e.g., adherent stem cells, e.g., grown on tissue culture plates) is limited by the surface area of the culture vessel. Thus, scalable growth and maintenance of stem cells is challenging.

SUMMARY

[0003] There is an unmet need for growth and maintenance of stem cells in suspension without the need for using expensive growth factors. The methods and systems disclosed herein use light-activatable domains to activate signaling pathways necessary for growing and maintaining stem cells, thereby meeting this unmet need.

[0003a] In one aspect, a method of growing and/or maintaining eukaryotic cells in suspension is provided, the method comprising: (a) culturing at least one eukaryotic cell in a culture media in suspension, wherein the at least one eukaryotic cell has been genetically engineered to express a fusion protein comprising a signaling protein receptor, or a functional portion thereof, and a light-activatable domain; (b) exposing the at least one eukaryotic cell to light to activate the light-activatable domain resulting in activation of a downstream signaling pathway of the signaling protein receptor, such that the at least one eukaryotic cell is grown and/or maintained in suspension.

[0004] In one aspect, a method of growing and/or maintaining stem cells in suspension is provided, the method comprising: (a) culturing the stem cells in a culture media in suspension, wherein the stem cells have been genetically engineered to express a first fusion protein, the first

fusion protein comprising a first signaling protein receptor or a functional portion thereof and a first light-activatable domain; and (b) exposing the stem cells to light thereby activating the first light-activatable domain resulting in activation of a downstream signaling pathway of the first signaling protein receptor, such that the stem cells are grown and/or maintained in suspension. In some cases, the signaling protein receptor or functional portion thereof is a growth factor receptor. In some cases, the culture media is deficient in one or more factor required for growing and/or maintaining the stem cells. In some cases, the one or more factor is a ligand for the first signaling protein receptor or functional portion thereof. In some cases, the one or more