https://stemcultures.com/

SUSTAINED RELEASE GROWTH FACTOR DELIVERY WITH DISC™ DEVICES FOR REPRODUCIBLE HIGH-QUALITY 3D CULTURES

OVERVIEW

Conventional human pluripotent stem cell (PSC) culture for organoid production requires a rigorous workload, including frequent medium change and technical clean-up of unwanted cells showing spontaneous differentiation. Homogenous PSC cultures are imperative: the quality of the originating PSCs impacts the efficiency and quality of downstream organoid differentiations. It is common to create 3D cultures from multiple PSC lines for comparison using parallel cultures. This can be challenging as lines from different donors often grow differently, leaving scientists struggling to maintain consistency across all lines. The quality of the 3D cultures generated typically cannot be determined until several weeks after production has begun, resulting in unnecessary labor and material waste when poor quality organoids result.

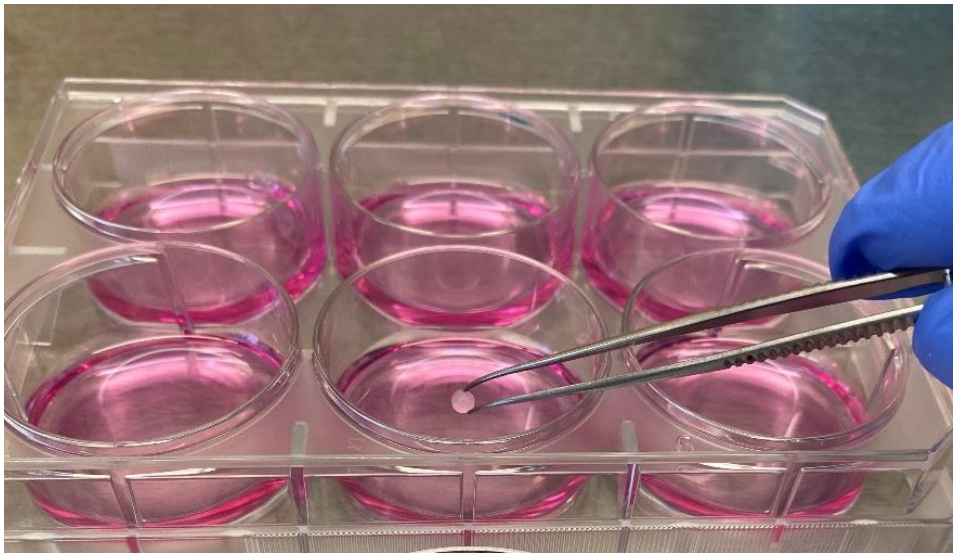

Figure 1. Image of An FGF2 DISC™ Device Being Added to a Well of a 6 Well Culture Plate.
The DISC provides one week of sustained-release FGF2, enabling reduced media exchanges and improved culture quality.

This document describes StemCultures DISC™ technology (**Figure 1**) that resolves these issues by improving the quality and consistency of PSC-organoid cultures. The DISC™ device applies controlled-release technology for cell culture to maintain stable growth factor concentrations and ratios over time. One DISC™ added to a culture releases the required growth factors at defined concentrations for 1-2 weeks (or until the next passage). DISC™ devices are composed of a non-degradable, mechanically robust, and inert hydrogel. Cells do not attach to the DISC™ device, and the DISCs do not directly interact with the cells in culture. DISCs are compatible with any culture medium and can be designed to fit any culture format. Users can change the culture media without removing the DISC device, and the DISC can be cleanly removed to stop growth factor exposure as needed.

SUSTAINED RELEASE OF FGF2 TO CREATE BETTER 3D CULTURES

Fibroblast growth factor 2 (FGF2) is a critical signal for maintaining high quality PSCs. FGF2, like many other growth factors, has a short half-life and decays within hours of being added to culture medium. As a result of FGF2 lability, stem cell scientists frequently replenish soluble FGF2 by daily feeding, which results in large fluctuations in FGF2 concentration (**Figure 2**). These fluctuations expose cells to a changing pattern of signaling which negatively impacts the maintenance of pluripotency and increases unwanted spontaneous differentiation. This type of stress affects PSC lines to unpredictably introduce heterogeneity between cultures. FGF2 DISCs™ address these limitations by stabilizing the level of FGF2. Using FGF2 DISCs™, scientists can obtain a new level of control over the PSC cultures environment to obtain homogeneous PSC cultures critical to improve the quality and robustness of 3D organoids.

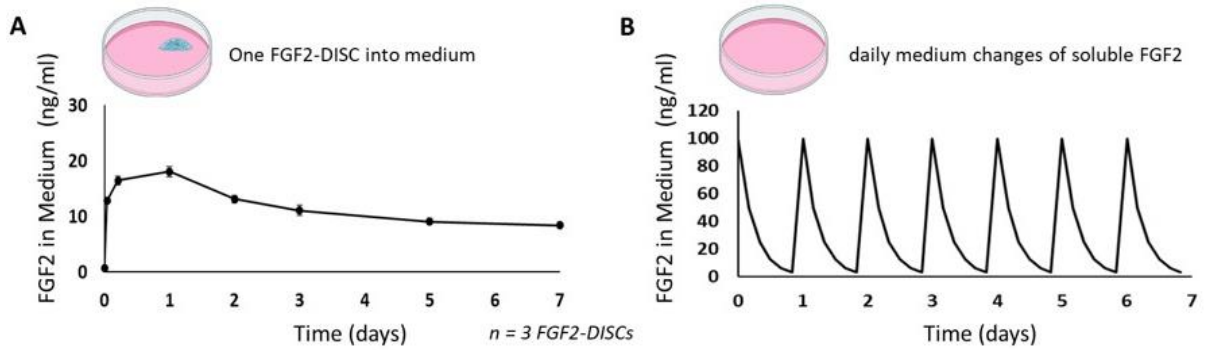

Figure 2. FGF2 DISC™ Use Maintains FGF2 Levels Without the Unstable Growth Factor Levels Seen Without Using Discs . A) Stable concentration of FGF2 delivered to PSC cultures by an FGF2 DISC™ device over seven days; B) Manual, daily replenishment of soluble FGF2 in commercial media formulations (100 ng/mL) during PSC medium changes results in highly unstable FGF2 levels.

DISC™ DEVICES IMPROVE PLURIPOTENCY AND REDUCE UNWANTED DIFFERENTIATION.

Using FGF2 DISC™ devices increases pluripotency markers (OCT4, SOX2, and NANOG) and decreases unwanted differentiation markers (SSEA-1) in PSC cultures when compared to daily feeding with commercial medium containing soluble FGF2 (**Figure 3**). As shown, there is significant correlation between a sustained FGF2 level and improved PSC pluripotency with reduced spontaneous differentiation.

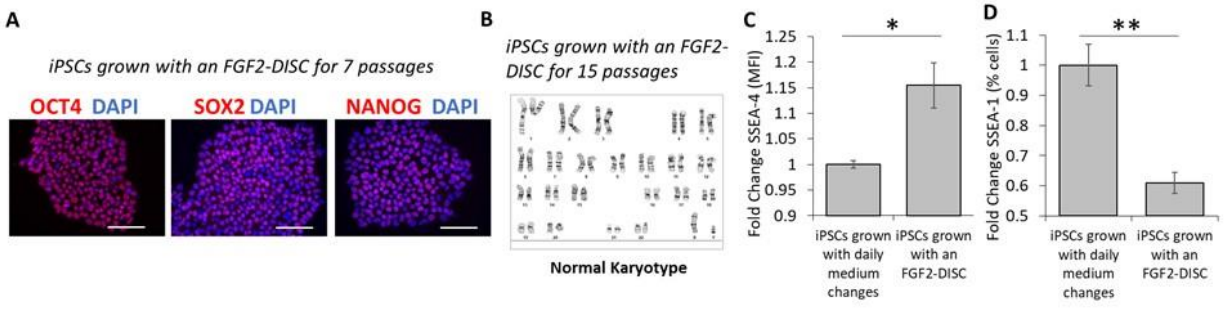

Figure 3. PSCs Cultured With an FGF2 DISC™ Show Improved Pluripotency.
A) PSCs grown with an FGF2 DISC™ stain positively for pluripotency markers after seven passages and B) show a normal karyotype after 15 passages. PSCs grown with the FGF DISC™ device for seven passages express higher levels of the pluripotency marker SSEA-4 (C) and lower levels of the undesired differentiation marker SSEA-1 (D) than do cells cultured with daily media exchanges (results for (C) and (D) obtained by flow cytometry).

DISC DEVICES REDUCE FEEDING, SAVING MEDIA AND LABOR EXPENSE.

With the addition of just one FGF2 DISC™ per passage, the cell culture environment is controlled, pluripotency is increased, and labor and media use are reduced. **Figure 4** shows a conventional daily feeding schedule compared to the simplified feeding schedule with an FGF2 DISC™.

DISCs work in your favorite culture medium, with fewer feedings. FGF2 DISCs™ save time and media usage while significantly improving the quality of the cell cultures produced.

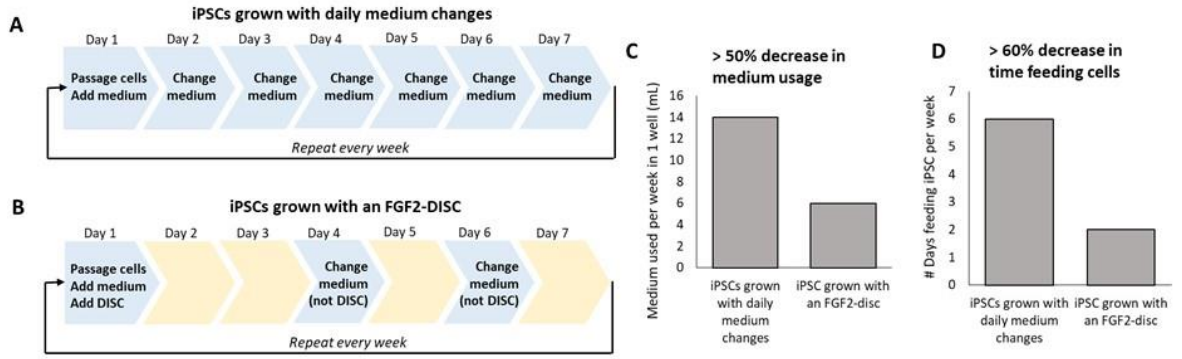

Figure 4. Use of FGF2 DISCs™ Reduces the Frequency of Media Changes during PSC Culture. The reduced feeding schedule provides substantial savings in time and medium use.

HOW DISCs™ MAINTAIN BETTER ORGANOIDS

The higher the quality of the initial PSCs, the higher the quality of organoids created.

Organoids grow well when the cells used to create them grow as a homogeneous, undifferentiated PSC culture. FGF2 DISC™ devices inhibit spontaneous differentiation to produce more homogeneous undifferentiated cell colonies. The heightened level of PSC quality achieved by stabilizing FGF2 levels using FGF2 DISCs™ more efficiently generates brain organoids of the highest quality (**Figure 5**).

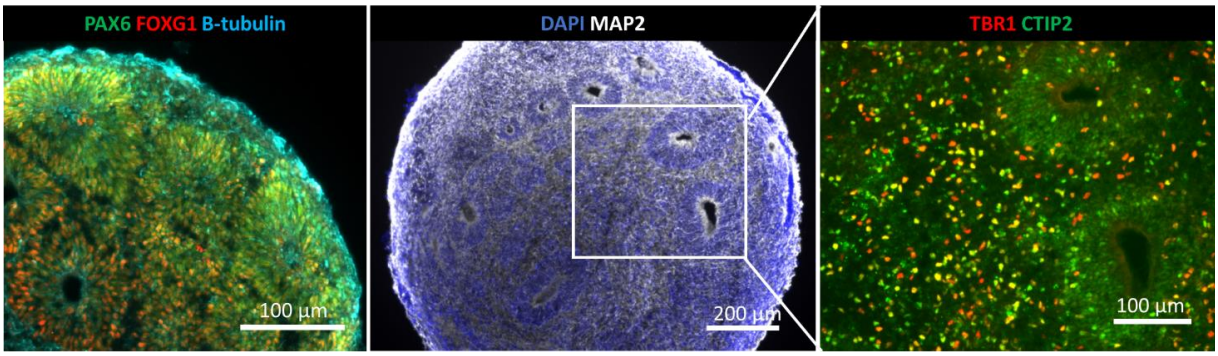

Figure 5. DISC™ Usage Improves the Efficiency and Reproducibility of Brain Organoid Production. Brain organoids generated from PSCs cultured with FGF2 DISCs are composed of progenitor cells in rosettes expressing PAX6 and FOXG1 after 20 days (A) and at 2 months, organoids are composed of robust, mature, MAP2 positive neurons (B) including deep layer cortical neurons expressing CTIP2 and TBR1 (C).

After organoid growth is initiated, the DISC™ technology continues to provide a stable growth factor environment during the differentiation process. Brain organoids may be grown for months with brain

derived neurotrophic factor (BDNF) and glial cell-line derived neurotrophic factor (GDNF). BDNF- and GDNF-containing DISC™ devices are available to maintain levels of these key survival and differentiation factors. Hence, specialized DISCs™ are used during the initial PSC growth and expansion, during the transition into organoids, and to support organoid development and maturation.

ASK US ABOUT DISC™ DEVICES CONTAINING YOUR FAVORITE GROWTH FACTORS

The possibilities for DISC™ devices to aid 3D cell culture are limitless with sustained release of different growth factors creating the most robust and reproducible growth factor environment for organoid production. In addition to the commercially available FGF2 DISC™, StemCultures produces custom growth factor DISC™ devices to benefit specific types of 3D cell culture, please contact us to describe your sustained-release growth factor needs.

ABOUT STEMCULTURES LLC

StemCultures develops novel reagents that improve the quality and efficiency of cell growth and differentiation in culture. The company's StemBead® and DISC™ devices support production of large, homogeneous quantities of cells through precise control of the culture environment, including the concentration and timing of the growth factors regulating cell proliferation and differentiation. This portfolio of products has proven value for stem cell cultures to reduce spontaneous differentiation, enhance pluripotency and guide preferential differentiation. For more information, please visit www.stemcultures.com.

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