StemCultures

STEMBEADS FGF2®

APPLICATION: Pluripotent Stem Cells Feeder Based Cultures

DESCRIPTION

StemBeads ® FGF2 is a patented growth factor supplement that offers a novel way to grow FGF2-dependent cell cultures more efficiently—*with fewer media changes*. StemBeads ® FGF2 contains an FDA-approved PLGA polymer loaded with FGF2 to release at a particular concentration. The stable level of FGF2 in culture allows for a more homogenoues, undifferentiated stem cell culture and while saving researchers valuable time with less media changes. Under the microscope, StemBeads ® appear as dark circles that do not harm the cells and break down over time.

PRODUCT DATA

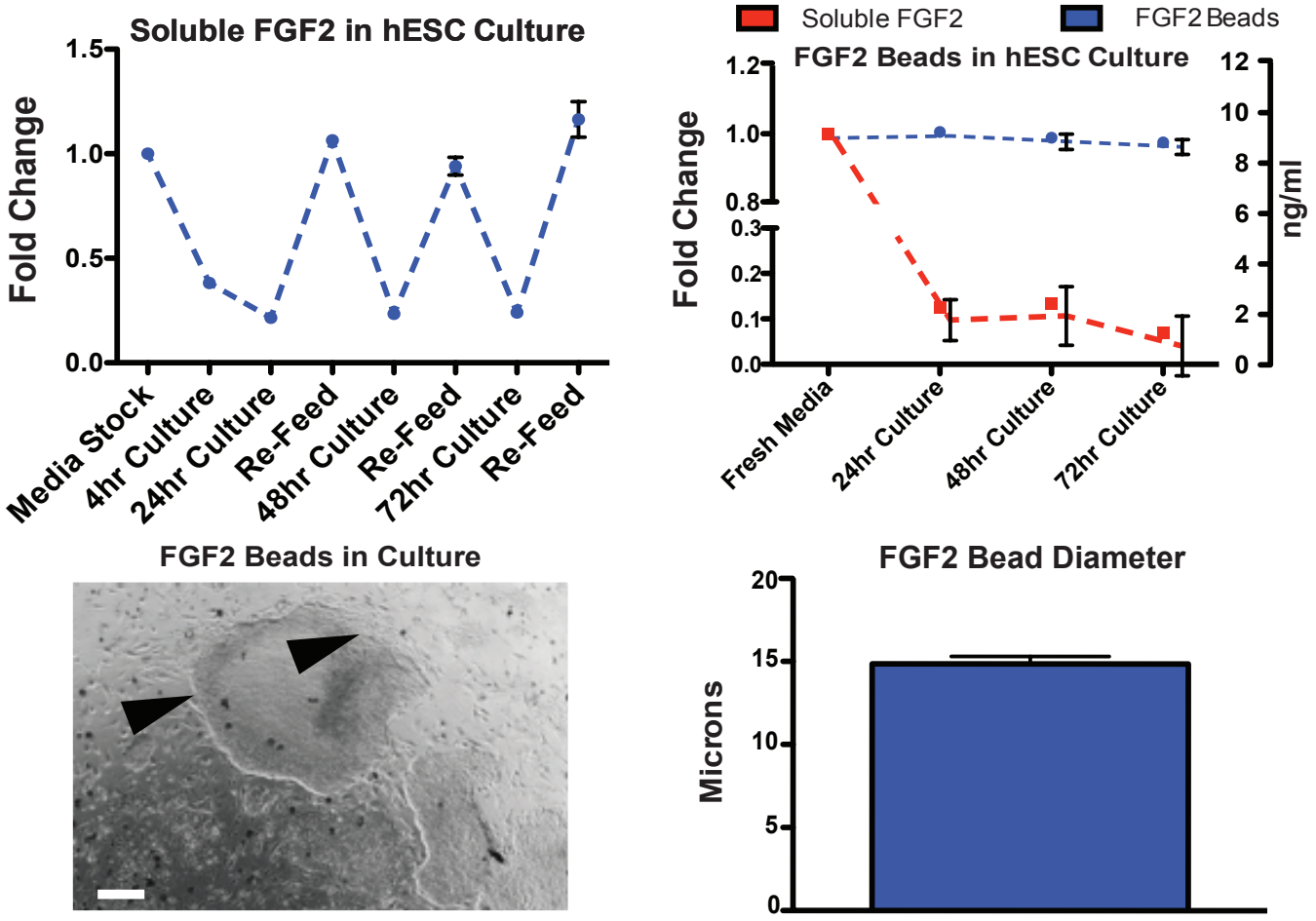

Figure 1. Sustained levels of FGF2 are achieved using StemBeads®

Standard daily medium changes in hESC culture lead to drastic fluctuations in FGF2 levels due to poor FGF2 stability. Using StemBeads ®, FGF2 is released at a constant rate over a three-day period sustaining FGF2 levels. FGF2 beads visualized in cultures (arrows) have an average diameter of 14.5 microns. Scale bar = 100 microns. (D) Using PLGA microspheres, FGF2 is released at a constant rate over a 3 day period, sustaining FGF2 levels.

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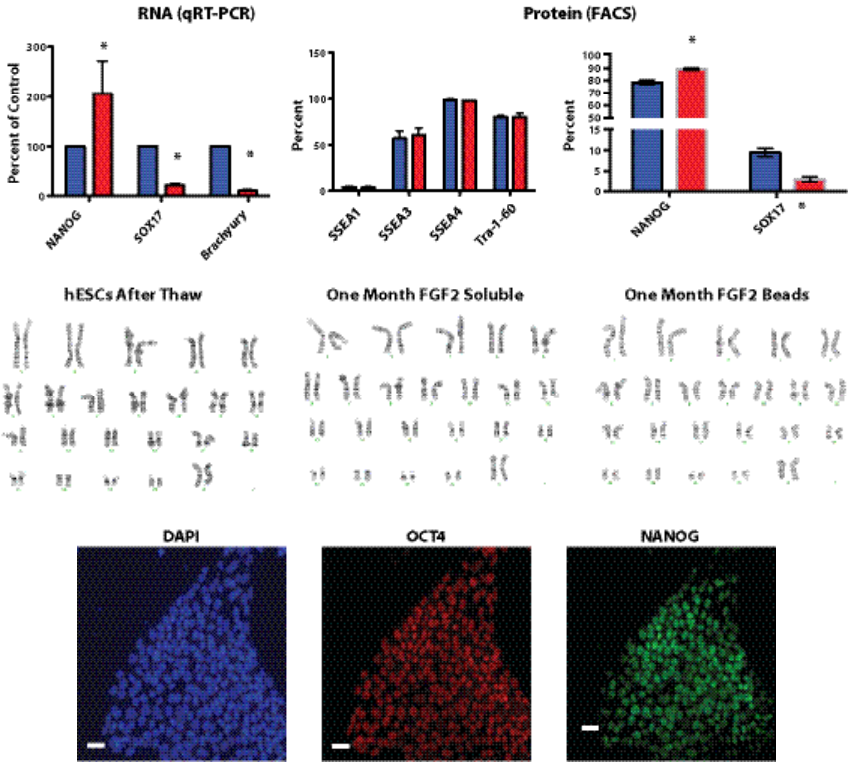
Figure 2. Culture with StemBeads ® and bi-weekly feeding produces a more undifferentiated hESC culture than traditional daily feeding

hESCs grown one month on MEF feeders with standard, KOSR media (Thermo) exhibit a similar FACS stem cell profile when fed with FGF2 beads in medium every third day compared to soluble FGF2 in medium daily. However, the expression of NANOG was significantly increased and the differentiation markers SOX17 and Brachyury were significantly reduced, by qRT-PCR (left panel). Normal Karyotypes for hESCs were assessed before expansion and then after one month of expansion in either soluble FGF2 or FGF2 beads, and neither showed abnormalities. Immunostaining of month-old cultures shows similar appearance of colonies and expression of the pluripotency markers OCT4 and NANOG in both conditions, Scale = 50 microns.



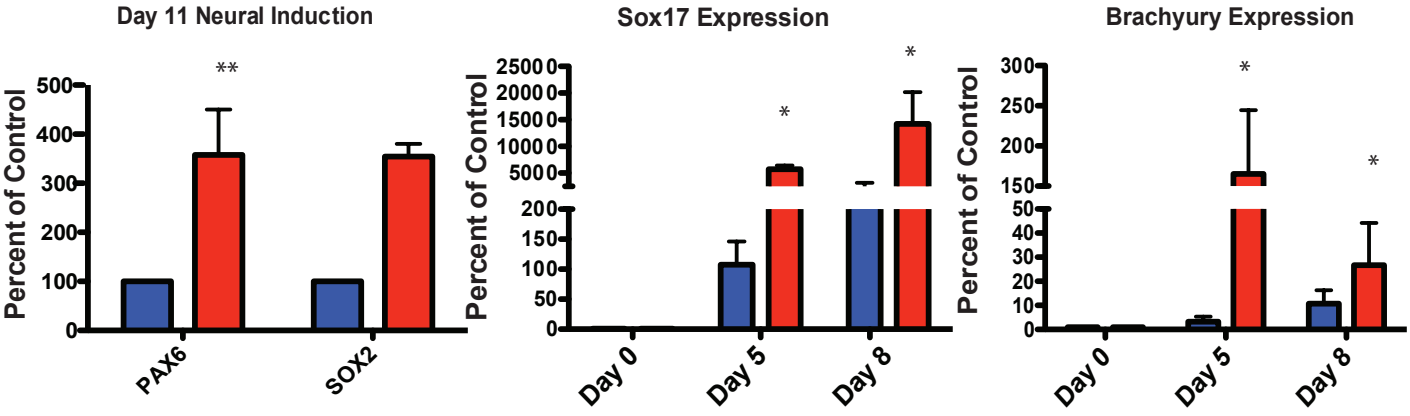


Figure 3. StemBeads ® FGF2 increase differentiation potential of hESC
Using directed differentiation protocols, hESC differentiation potential was assessed for all three germ lineages. From left to right panels, hESCs grown using FGF2 beads gave rise to neural, mesodermal, and endodermal progeny, assessed by qRT-PCR for relevant markers. StemBeads ® FGF2 expanded hESCs expressed higher levels of lineage markers after differentiation to all lineages.

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Application Notes

Using StemBeads® FGF2 for Maintenance and Expansion of hPSCs in feeder conditions

Product Handling / Directions For Use

Reconstitution & Use:	StemBeads® FGF2 are provided as a ready-to-use 3mL solution in DMEM/F12
Storage & Stability:	Upon arrival store at 4°C. StemBeads® FGF2 are stable for up to 6 months without loss of activity when stored at 4°C.
Release Profile:	8µL/mL Stembead® FGF2 = 10 ng/mL release of soluble FGF2
Physical Characteristics:	StemBeads® FGF2 are 12 ± 1um in diameter.

For further details concerning StemBeads® FGF2, please refer to StemBeads® FGF2 Product information Sheet.

Suggested Protocol

The following protocol describes a procedure that entails splitting once a week and feeding 2-3 times a week for the maintenance and/or expansion of hPSCs on feeders. Please note that there may be a slight adjustment period for the first two passages as the cells transition. Additional optimization may be required due to variability between hPSC lines.

Preparation of Media with StemBeads-FGF2:

1) Aliquot desired volume of defined hPSC media.
2) Mix vial of StemBeads® FGF2 thoroughly by vortexing or pipetting prior to use as the beads will settle quickly.
3) Add StemBeads® FGF2 into aliquot of defined media at a concentration of 8uL StemBeads® FGF per 1mL of media for a 10ng/ml release.

Culturing hPSCs with StemBeads-FGF2:

Day -3:	Remove media and replace with freshly prepared StemBeads® FGF2 supplemented media.
Day 0:	Passage hPSCs using preferred enzymatic treatment, then re-plate the hPSC in media supplemented with StemBeads® FGF2 onto mouse embryonic feeders.
Day 1 *(Optional)*:	If a large amount of unattached/dead cells are observed, wash 2x with DMEM/F12* and replace with freshly prepared StemBeads® FGF2 supplemented media.
Day 4:	Remove media, wash 2x with DMEM/F12* and replace with freshly prepared StemBeads® FGF2 supplemented media.
Day 7:	Repeat splitting and feeding as described above.
	***Note:** Washing is highly recommended prior to every feed to remove cell debris and excess beads

References

Lotz S., Goderie S., Tokas N., Hirsch S.E., Ahmad F., Corneo B., Le S., Banerjee a., Kane R.S., Stern J., Temple S., Fasano C.A. Sustained Levels of FGF2 Maintain Undifferentiated Stem Cell Cultures with Biweekly Feeding. *PLoS ONE* 2013, 8 (2)

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