Qkine

Bioactive thermostable (heat stable) FGF2-G3 growth factors for iPSCs cultures

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The challenge

Induced pluripotent stem cells (iPSCs) can proliferate indefinitely. As their differentiation can be directed towards derivatives of all three germ layers, they have a broad spectrum of applications, ranging from drug discovery to regenerative medicine.

There are both technical and cost implications associated with the culture of iPSCs which are mostly due to the frequent media changes required. This labour-intensive approach significantly increases the average time and effort to maintain the efficiency of these cultures when compared to other common cultures. Widely adopted media for the culture of iPSCs, including mTeSR, StemPRO and E8, require daily media changes to maintain the cell's morphology, proliferative ability, and differentiation capacity. This is a result of inherent instability and proteolytic degradation of one of the key components, fibroblast growth factor-2 (FGF-2), which is an essential growth factor for regulating several biological processes and maintaining stem cell pluripotency. This fundamental biochemical instability and short half-life in culture media (<10 h) can affect the health status of iPSCs, homogeneity and subsequent differentiation capacity.

Notably, these widely adopted media for the culture of iPSC cells are very costly, and frequent media changes indicate a further increase in the cost of culture maintenance. Therefore, there is an urgent need for a suitable cost-effective, efficacious, and stable alternative for the culture and maintenance of iPSCs.

Technological solution - FGF2-G3

Qkine provides high-quality and bioactive growth factors produced in animal-free systems. Qkine FGF2-G3 (Qk053) is a high purity 17 kDa bioactive protein with a 154 aa mature domain of FGF-2. This protein has nine amino acid substitutions to enhance stability without impacting bioactivity (Dvorak et al. 2018), which increases the functional half-life to >7 days. This patented technology from Enantis (FGF2-STAB) has been licensed by Qkine and combined with manufacturing expertise to generate an animal-free, tag-free, thermostable recombinant protein suitable for stem cell culture process development and scale-up. The ability to engineer a thermostable form of FGF-2 with an increased functional half-life from <10 h (wild-type) to >7 days (FGF2-G3) creates a unique opportunity to extend the stability of relevant media. B8 media (Kuo et al., 2019) and other chemically defined stem cell culture media, can be used in conjunction with FGF2-G3 for weekend-free, highly homogeneous, induced pluripotent stem cell culture.

The scientific approach

Thermostable FGF-2 (FGF2-G3) was used to examine its biological effects on iPSC cultures. The stability of FGF2-G3 in conditioned media for 72 hours was examined by comparing it to the wild type (WT) FGF-2 (Fig. 1). Furthermore, the viability, morphological characteristics, and pluripotency of iPSCs cultured in FGF2-G3 was also assessed by directly comparing its effect to mTeSR and Stem Flex media. (Fig. 2 and 3)

Results

Fig.1

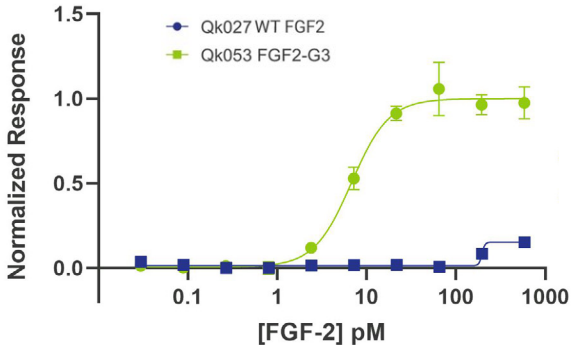
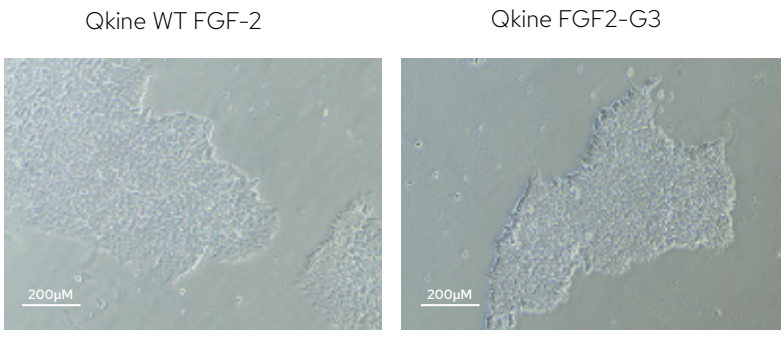


Fig.2



Images represent:
(1) Qkine WT FGF-2 (Qk027) and Qkine thermostable FGF2-G3 (Qk053) bioactivity in conditioned media for 7 days.
(2) Morphological and viability assessment of WT iPSCs cultured in mTeSR with Qkine FGF-2 and thermostable FGF2-G3 for 3 days. Scale bar 200µM. Images taken with Nikon Eclipse Ts2 Microscope, 10x Air Objective (NA=0.3), DS-Fi3 Color Camera.

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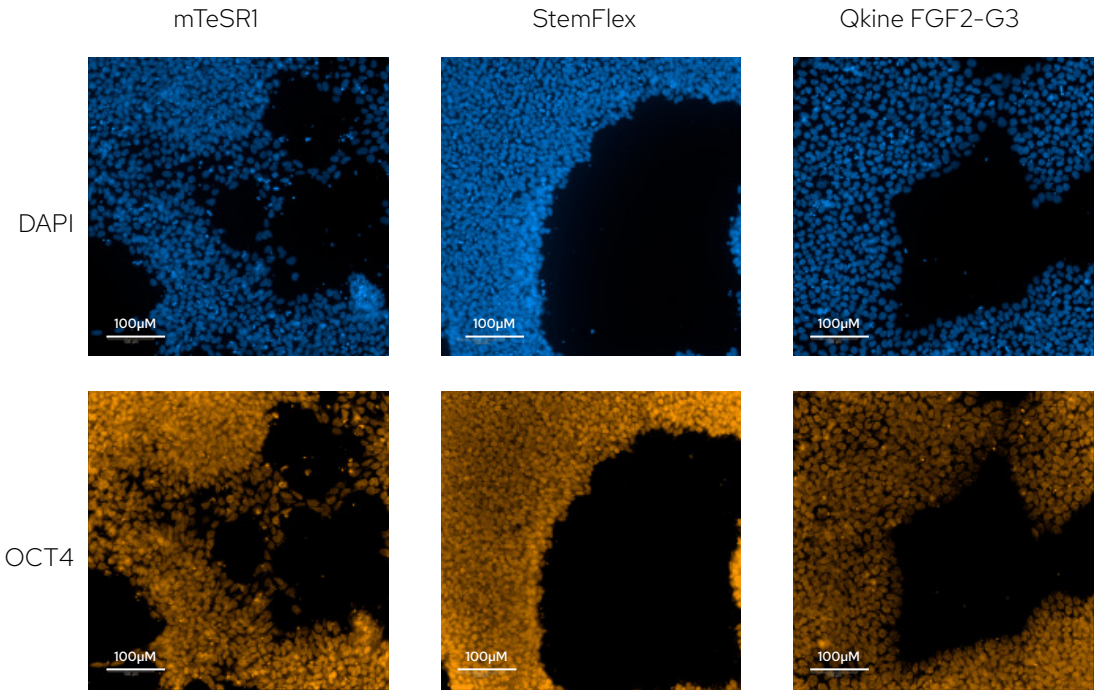
Results continued

Fig.3



(3) Proliferation and pluripotency assessment of WT iPSCs cultured in mTeSR1, StemFlex and mTeSR1 + Qkine FGF2-G3 for 4 days without a media change. Scale bar 100µM. Images taken on Operetta CLS using 20x Water Objective (NA=1.0). Analysis performed in Harmony v4.9 software.

Discussion and future work

Findings from this study showed a significant reduction of WT FGF-2 bioactivity when used in culture for 7 days while Qkine thermostable FGF2-G3 retained its bioactivity for the same duration. Thermostable FGF2-G3 maintained the iPSCs proliferative state with enhanced colony health when compared to WT FGF-2, without the need for constant media changes.

Thermostable FGF2-G3 maintained pluripotency with fewer media changes than Stem Flex thermostable FGF-2, based on pluripotency marker OCT4 expression level. Taken together, Qkine thermostable FGF2-G3 offers an improved stable version of FGF-2 for enhanced cost-effective stem cell cultures. Future work will aim to further characterize iPSC cultures used with thermostable FGF2-G3.

> "Qkine FGF2-G3 was really easy to substitute into our experiments and offers a stable solution to iPSC and FGF-2-dependent cultures. It enabled us to save time and money while maintaining the quality of our cells."
>
> Felix Buchner and Joshua Thomas
> DZNE, Germany

To find out more about this product and any of our other products, visit www.qkine.com or scan this QR code on your smartphone.

References

Dvorak P, Bednar D, Vanacek P, et al. (2018) Computer-assisted engineering of hyperstable fibroblast growth factor 2. *Biotechnol Bioeng*. 115(4):850-862.

Kuo HH, Gao X, DeKeyser JM, et al. (2019) Negligible-Cost and Weekend-Free Chemically Defined Human iPSC Culture. *Stem Cell Reports*. 14(2):256-270.

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CSFGF2-170423-v1.2

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