

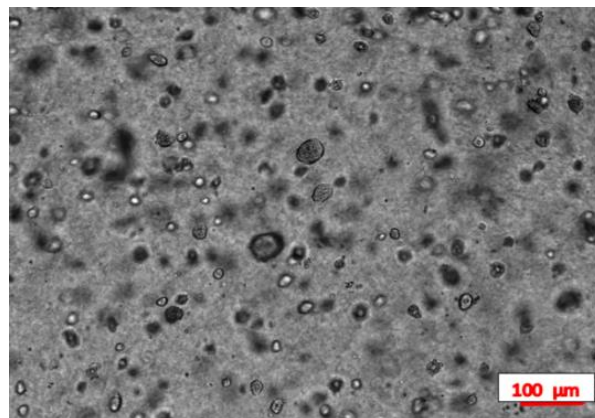
## hiPSC-qualified PGmatrix™-case study

PGmatrix-hiPSC is a fully-defined, peptide-based hydrogel. When used in combination with PGworks and PGgrow-hiPSC, the 3D system can successfully support the growth of different human induced pluripotent stem cell (hiPSC) lines using mTeSR1 medium.

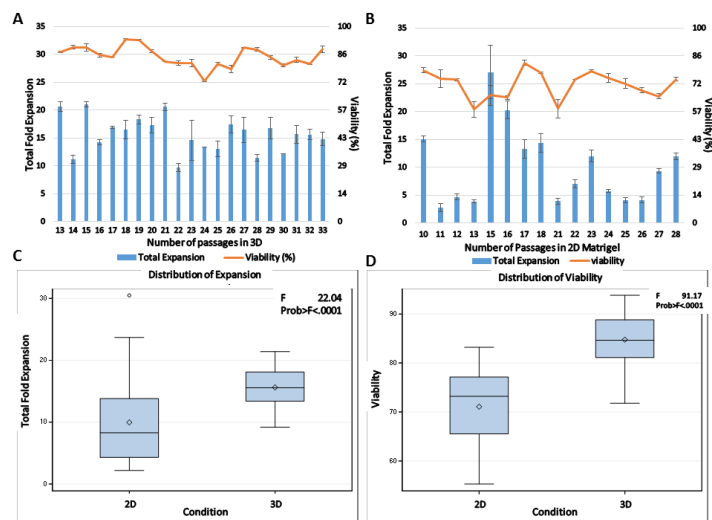
**3D encapsulation and culture in PGmatrix-hiPSC:** hiPSCs harvested from 2D (Matrigel or Vitronectin XF-coated plates) or 3D (PGmatrix-hiPSC) were encapsulated in 0.5% PGmatrix-hiPSC following the procedure described in “hiPSC-qualified PGmatrix™ using guide”. The seeding density ranges from  $1.8 \times 10^5$  cells/mL to  $3 \times 10^5$  cells/mL. The mTeSR1 complete medium supplemented with PGgrow was changed every day starting from Day 2 culturing. hiPSCs have been cultured continuously in 3D PGmatrix-hiPSC for more than 30 passages (passaged every 5 days).

**Results Summary:** 3D PGmatrix-hiPSC provides *in vivo*-like scaffolds for hiPSCs. When encapsulated, cells grown into spherical colonies (**Figure 1**). In this 3D environment, cells adapt to it easily, start rapid population expansion on Day 3 and reach peak population number and viability around Day 6. Cell growth in this 3D system shows better stability in total hiPSC population expansion and cell viability than that in conventional 2D systems. The average total expansion as harvested on Day 5 is about 15 times for hiPSC line (**Figure 2**) and 8 times for another hiPSC line (data not shown). Long term culture of hiPSCs in 3D PGmatrix-hiPSC reaches higher or similar population expansion compared with 2D culture with Matrigel or Vitronectin-XF, while the 3D system uses 80% less space and 50% less culture medium. Another advantage of PGmatrix-hiPSC is that it allows culture fixation and immuno-staining to be performed directly in 3D. **Figure 4** is an example of hiPSC 3D culture stained with Oct3/4 antibody (green).

**Figure 1.** Typical hiPSC morphology in 3D PGmatrix-hiPSC system (Day 3)



**Figure 2.** Population expansion between different passages of hiPSC line in 3D PGmatrix-hiPSC and 2D Matrigel



**Figure 3.** hiPSCs stained for Oct4 (green) directly in 3D PGmatrix-hiPSC

