Supplementary Information:

Materials and Methods:

Immunofluorescence staining of feeder cells to detect Collagen IV

Cells were fixed with Mildform 20 N (8% formaldehyde, pH 7.0–7.5; Wako Pure Chemical Industries) for 30 min and permeabilized with 0.2% Triton X-100/PBS for 2 min. After blocking with 1% BSA solution for 30 min at room temperature, the samples were further processed as required. For processing of feeder cells for collagen-IV immunostaining the chemically fixed HDFs were washed thrice with Hanks' Balanced Salt Solution (HBSS, Life Technologies, Gibco, CA) and incubated with Collagenase Type IV (Life Technologies, Gibco, CA 50 U/ml in HBSS) at 37°C for 4 h. After this the chemically fixed HDFs were washed using HBSS thrice and exposed to rabbit polyclonal to Collagen-IV (Abcam) followed by incubation with an Alexa Fluor 488-conjugated Goat anti-rabbit polyclonal secondary antibody (Abcam).

Results and Discussion:

Collagen-rich ECM retained and preserved in chemically fixed feeder cells

Collagen-IV, an ECM component which is essential for stem cell adhesion and growth[16] was detected in both live, and chemically preserved chemically fixed autologous feeders (**Figure S1**: A-D). The chemical fixation technique cross linked and preserved this collagen-rich ECM and resisted degradation when treated with Collagenase (**Figure S1**: G, H) compared with non-fixed samples (**Figure S1**: E, F). Since the chemically fixed feeder cells supported human iPS culture, we concluded that essential ECM proteins, such as Collagen IV is required for iPS cell adhesion. In vivo, stem cells reside in their own anatomic niches in a defined physiological environment which also provides nourishment to the cells[14]. Physically the niche also serves to anchor the stem cells by various ECM components and cell-adhesion molecules[14]. Therefore in-vitro culture on chemically fixed feeder that retain ECM proteins closely resemble in vivo stem cell niches in functionality[14]. In line with this observation, several in vitro stem cell culture protocols have been developed that replace the use of traditional feeder cells by protein rich matrices such as laminin[16], collagen IV/fibronectin/laminin[18], and vitronectin[27]. It should be noted that, in many of these matrix conditions, the use of fibroblast-conditioned medium or defined medium such as mTeSR™ is a must to supplement the proteins missing from the culture matrices but are deemed necessary for culture. We are of the opinion that chemical fixation captures all of the necessary ECM proteins (one of which is shown here: Collagen IV, **Fig. S1**) and retains them in the cross linked feeder matrices. Therefore this method provides more feasible and a safer clinical approach for iPS cell culture on multicomponent protein matrices compared to culture atop Matrigel™ which is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm mouse sarcoma, rich in extracellular matrix proteins[16].

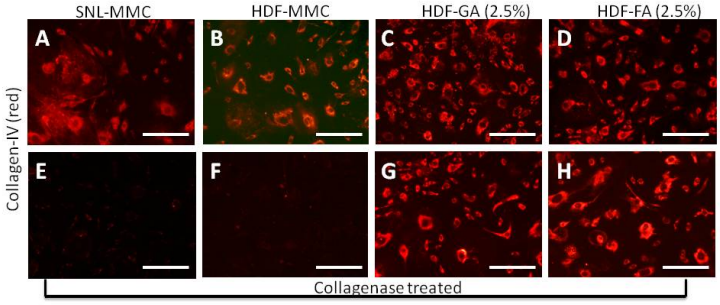

Figure S1. Immunofluorescence staining of Collagen IV in mitotically fixed and chemically fixed feeder cells (A-H). Scale bars, 100 µm in all images.

When feeder cells were treated with Collagenase prior to staining, the chemically fixed cells retained the Collagen IV (G, H), but not the others (E, F).