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A Serum-Free Chemically Defined Medium for the Expansion of Adult Normal Human Dermal Fibroblasts

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Introduction

Dermal fibroblasts are a vital component of the skin, producing various cytokines and chemokines that promote the growth of keratinocytes, affecting formation of extracellular matrices and promoting wound repair. *In vitro* expanded dermal fibroblasts are used clinically for acute and chronic wound repair and as a source of collagen for cosmetic surgery. Traditionally, *in vitro* cell culture medium contains animal-derived products like serum, tissue extracts or hydrosylates. They are also a source of undesirable factors, such as endotoxin, and pose potential BSE risks. The undefined and variable nature of these components is a challenge in basic research and applied biomedical sciences. Lonza has developed a serum-free chemically defined, fibroblast growth medium (TheraPEAK™ FGM-CD™ medium), to propagate primary adult normal human dermal fibroblasts (NHDF). The characteristics of NHDFs from several donors that were cultured in FGM-CD™ medium were compared with cells grown in serum-containing medium. The properties of NHDFs in FGM-CD™ medium grown on different plastic surfaces were also examined. Key factors of wound repair, such as cell migration and extracellular matrix production by dermal fibroblasts in FGM-CD™ medium, were also examined. Replacing serum-containing medium with TheraPEAK™ FGM-CD™ medium for the *in vitro* expansion of fibroblasts provides a more defined environment for pharmacological manipulations for research studies and for clinical applications using fibroblasts.

Materials and methods

Cell culture

Clonetics™ NHDFs (Lonza) were thawed and processed according to the published protocol. Cells were seeded directly in TheraPEAK™ FGM-CD™ medium or DMEM with 10% FBS (Lonza). NHDFs were plated either in T25 flasks, 6-well or 48-well plates, depending on the assays.

Cell proliferation

Population doublings were assayed from cells grown in flasks by trypan blue exclusion.

Immunofluorescence (IF) and flow cytometry (FACS)

For both direct IF and FACS, control antibodies used were mouse isotype IgG1κ (Sigma) and phycoerythrin (PE) mouse IgG1κ (BD Biosciences). The primary antibodies used were mouse monoclonal (5B5) to fibroblast and collagen I (Abcam) and PE mouse anti-human CD90 (BD Biosciences). The secondary antibody, goat anti-mouse Alexa Fluor® 555 (Invitrogen), was used to label 5B5 and collagen I. Briefly, for IF, cultured NHDFs in 48-well plates were fixed with methanol, washed with PBS and stained with primary and secondary antibodies. NHDFs collected from T25 flasks were fixed and permeabilized with paraformaldehyde and methanol, followed by staining with the antibodies. The expression of 5B5 and CD90 was measured using a FACSort™ flow cytometer and analyzed using CellQuestPro™ software.

Wound scratch and collagen assays

For the scratch assay, a confluent NHDF monolayer was wounded using a sterile 200 µl pipette tip. For baseline, the scratch was imaged immediately (0 hours) and after 24 hours at a reference point, using a Zeiss inverted light microscope. The wound healing was determined by measuring the gap of the scratch at the two time points using the Image J program.Collagen assays were performed using the picric Sirius Red method. Briefly, NHDFs grown to confluence were washed with PBS and pelleted. Cell pellets were incubated with 0.5 N acetic acid overnight at 4°C. 1 ml of dye reagent composed of picric acid and Sirius Red (Sigma) was added to the pellet or collagen standard, and mixed at room temperature for 30 minutes. After centrifugation for 5 minutes, supernatants were discarded. The bound dye was released with 0.5 M NaOH. The 200 µl solution was transferred to a 96-well plate and absorbance at 540 nm was read using SpectraMax® M5 microplate reader (Molecular Devices).

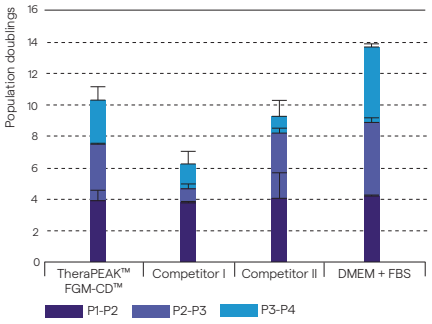

Figure 1A: NHDFs grown in TheraPEAK™ FGM-CD™ medium outperform the cells grown in competitors' serum-free dermal fibroblast media. The total population doubling yield was 75% to serum-containing medium (and significantly better than both competitors at passage 3, p <0.05). The fibroblasts were fed every 3 days and passaged at 80% confluence. Data shown is an average of three donors.

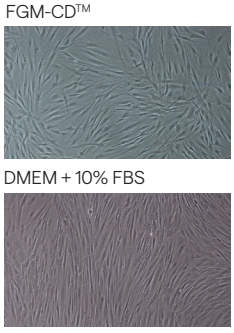

Figure 1B: Morphology of NHDFs in TheraPEAK™ FGM-CD™ medium. Cells grown in FGM-CD™ medium (shown here at passage 2, day 7) are spindle-shaped, a characteristic trait of fibroblasts.

Growth of NHDF in FGM-CD™ medium on different flask surfaces

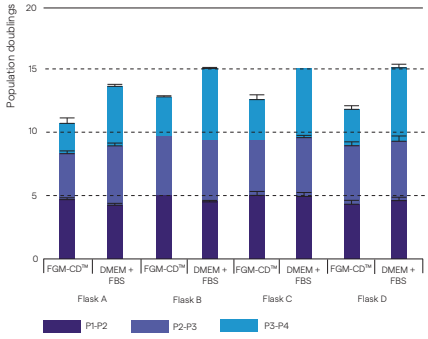

Figure 2: Population doublings of NHDFs grown in (A) TheraPEAK™ FGM-CD™ medium and (B) DMEM + 10% FBS on different T25 flask surfaces were assessed for 3 passages. Growth and morphology were similar across all surfaces.

Immunofluorescence staining of NHDF in FGM-CD™ medium

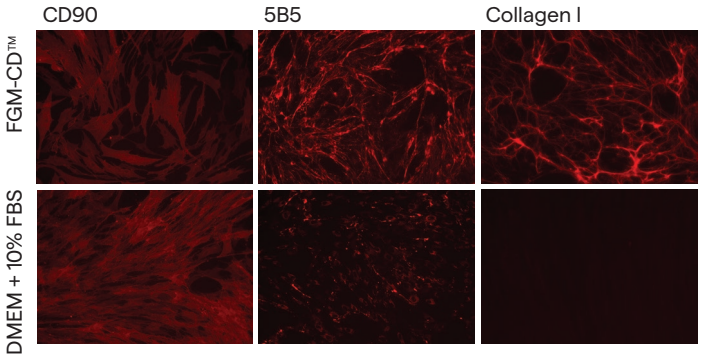

Figure 3: Representative immunofluorescence micrographs show similar expression of CD90 when grown in TheraPEAK™ FGM-CD™ medium or serum-supplemented medium. NHDFs show immunoreactivity to fibroblast marker 5B5 in both media. NHDFs in FGM-CD™ medium show marked increase in type I collagen.

Expression of CD90 and 5B5 by NHDF in FGM-CD™ medium

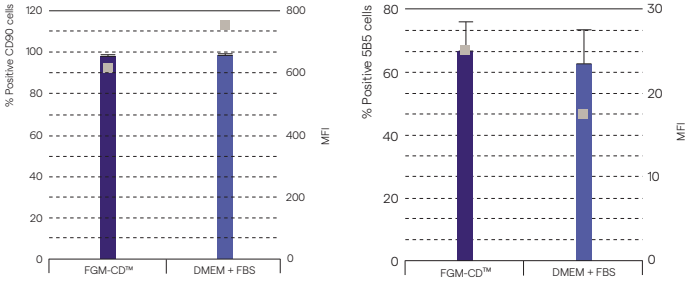

Figure 4: FACS analysis demonstrates that CD90 is positively expressed on NHDF in both serum-free and serum-containing media. The mean fluorescence intensity (MFI) of CD90 was slightly higher in DMEM + FBS. Expression of 5B5 was similar in both media, but MFI was slightly higher in FGM-CD™ medium.

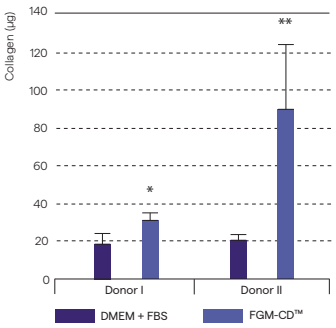

Figure 5: Collagen detection by the picric Sirius Red method. The collagen production in the NHDFs grown in FGM-CD™ medium is significantly greater than for cells grown in serum-containing medium (* p <0.05, ** p <0.001).

Collagen synthesis by NHDF in FGM-CD™ medium

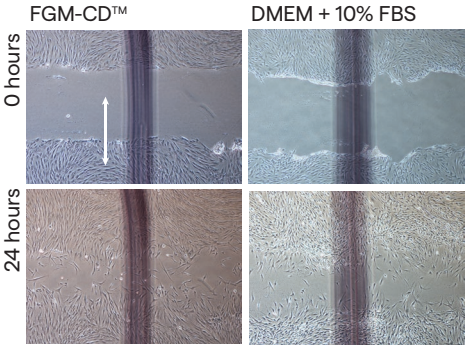

Figure 6A: Scratch assay micrographs: the wounded monolayer was imaged at time 0 hours and 24 hours and the distance measured at the reference point (arrow, dark lines).

Migration of NHDFs in a scratch assay

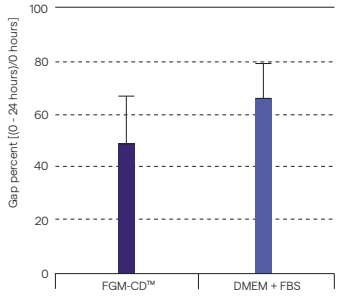

Figure 6B: Cell migration is shown by the gap percent calculated by the difference (0 - 24 hours)/ distance at 0 hours. There is no significant difference in migration between cells in FGM-CD™ medium and DMEM with FBS (n=3).

Conclusion

- TheraPEAK™ FGM-CD™ medium is a serum-free chemically defined medium that supports expansion of adult human dermal fibroblasts.
- The growth performance of NHDF in FGM-CD™ medium is 75% to DMEM with serum averaging 11 population doublings over 3 passages.
- NHDFs in FGM-CD™ medium express fibroblast markers similar to cells grown in DMEM-FBS.
- The fibroblasts grown in the chemically defined FGM-CD™ medium secrete collagen and maintain migration capability.
- Replacement of serum-containing medium with TheraPEAK™ FGM-CD™ medium for the *in vitro* expansion of fibroblasts provides a more defined environment for pharmacological manipulations for research studies and clinical applications using fibroblasts.

References

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 CD-SP067 11/23

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