Corning® U-shaped 75 cm² Flask Demonstrates Same Cell Growth as Traditional Rectangular Design

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SnAPPShots

A brief technical report from the Corning Applications Group

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Introduction

Corning's U-shaped 75 cm² flask incorporates improved design features that benefit the researcher without disrupting culture conditions. The rounded shoulders provide a better approach for rinsing the growth surface and easier access when scraping to collect cells. The U-shaped flask integrates a unique design for improved ergonomics.

Method and Materials

Each evaluation was repeated three independent times with n = 9 per vessel shape.

Evaluating Cell Growth Under Standard Conditions

HEK-293 (ATCC® Cat. No. 1573) and CHO-K1 (ATCC Cat. No. 9618) cells were seeded into rectangular shaped and U-shaped flasks at 5,000 cells/cm² and 3,000 cells/cm², respectively. Cells were cultured for 4 days in Corning DMEM (Cat. No. 10-013-CM) supplemented with 10% Corning FBS (Cat. No. 35-010-CV). Cells were harvested and enumerated using the Beckman Vi-CELL® viability analyzer.

Evaluating Cell Growth Under Reduced Serum Conditions

HEK-293 and M1WT2 (ATCC Cat. No. CRL-1984) cells were seeded into both rectangular and U-shaped flasks at 6,000 cells/cm² and 5,000 cells/cm², respectively. HEK-293 cells were cultured in DMEM with Corning 1X non-essential amino acids (Cat. No. 25-025-CL) containing 10% FBS. CHO-K1 cells were cultured in Corning F12K (Cat. No. 10-025-CV) containing 10% FBS. After overnight incubation at 37°C and 5% CO_2, the medium was exchanged with reduced serum medium containing 1% FBS. Cells were cultured for 2 additional days before harvesting and enumerating via the Vi-CELL viability analyzer.

Evaluating Cell Growth of Primary Cells

Primary human epidermal keratinocytes isolated from adults (HEKa) (Gibco Cat. No. C-005-5C) were plated at a concentration of 2.5 x10³ cells/cm² in 0.2 mL/cm² of Medium 154 supplemented with 1X human keratinocyte growth supplement (HKGS) (Gibco Cat. No.M-154-500 and S-001-5). The cells were cultured for 4 days with a medium change 2 days after plating. Cells were harvested and enumerated via the Vi-CELL viability analyzer.

Evaluating Neural Stem Cell Growth Exposed to Various Temperatures

Rectangular shaped and U-shaped 75 cm² flasks were coated with 4 µg/cm² of Poly-L-ornithine (Sigma® Cat. No. P3655) for 1 hour at 37°C. The flasks were then washed twice with cell culture grade water (Corning Cat. No. 25-055-CV) and coated for 2 hours at 37°C with 2 µg/cm² of Laminin (Sigma Cat. No. L2020). Finally, flasks were filled with 15 mL of Phosphate Buffered Saline (PBS) (Corning Cat. No. 21-031-CM) for up to 1 week at 4°C until ready for use. Upon use, PBS was removed and flasks were seeded with neural stem cells (NSCs) (Gibco® Cat. No. N7800-100) at 40,000 cells/cm² in complete neural stem cell medium (Gibco Cat. No. A105009-01). Medium was changed every other day for 4 days until cells were harvested. After enumeration, the cells were fixed and stained for multipotency analysis via the Miltenyi Biotech MACSQuant® flow cytometer. Cells were then permeabilized using a 0.1% Saponin solution (TCI Cat. No. S0019) and stained with two commonly used markers for multipotency, nestin (R&D Systems® Cat. No. IC1259F) and SOX2 (R&D Systems Cat. No. IC2018A).

Results and Discussion

Quality and consistency are extremely important for successfully culturing cells. When any aspect of an experiment is altered, it is important to thoroughly evaluate the change to ensure there are no unforeseen effects. In this study, we have demonstrated how various cell types including cell lines, primary cells, and stem cells perform the same regardless of whether they were cultured in traditional rectangular 75 cm² flasks or Corning U-shaped 75 cm² flasks. Figures 1 to 4 demonstrate no statistical difference between the two vessels for cell growth, adherence, or stem cell multipotency expression (Figure 5).

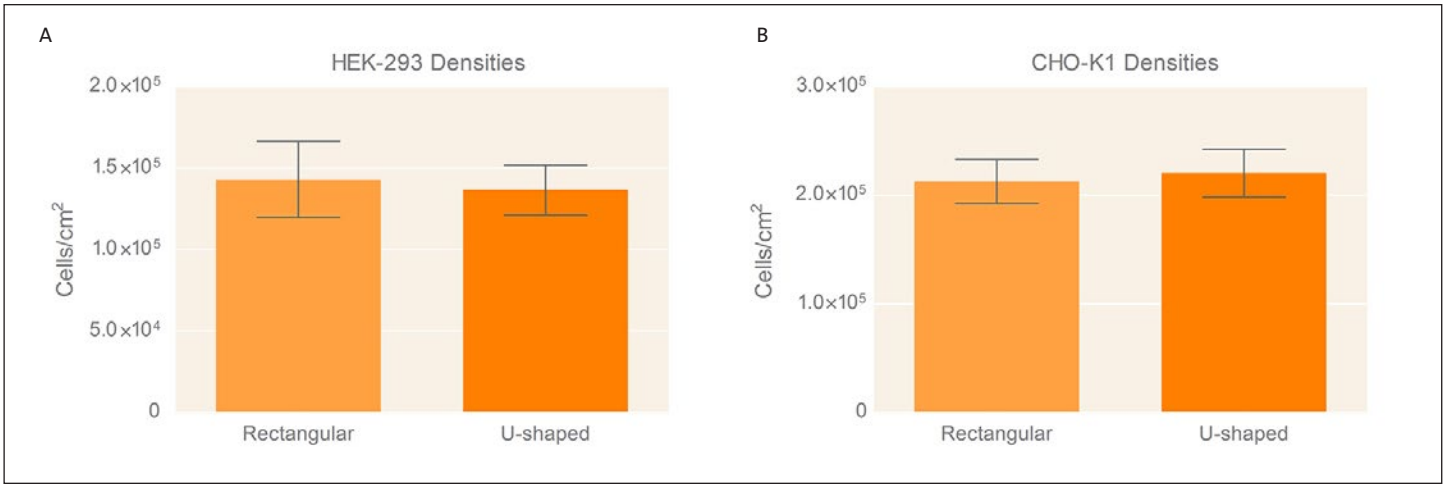

Figure 1. Similar cell growth was obtained under standard conditions. (A) HEK-293 and (B) CHO-K1 cells were cultured for 4 days after which cells were harvested and enumerated. No statistical difference was observed between both vessels. Paired T-test. n = 9.

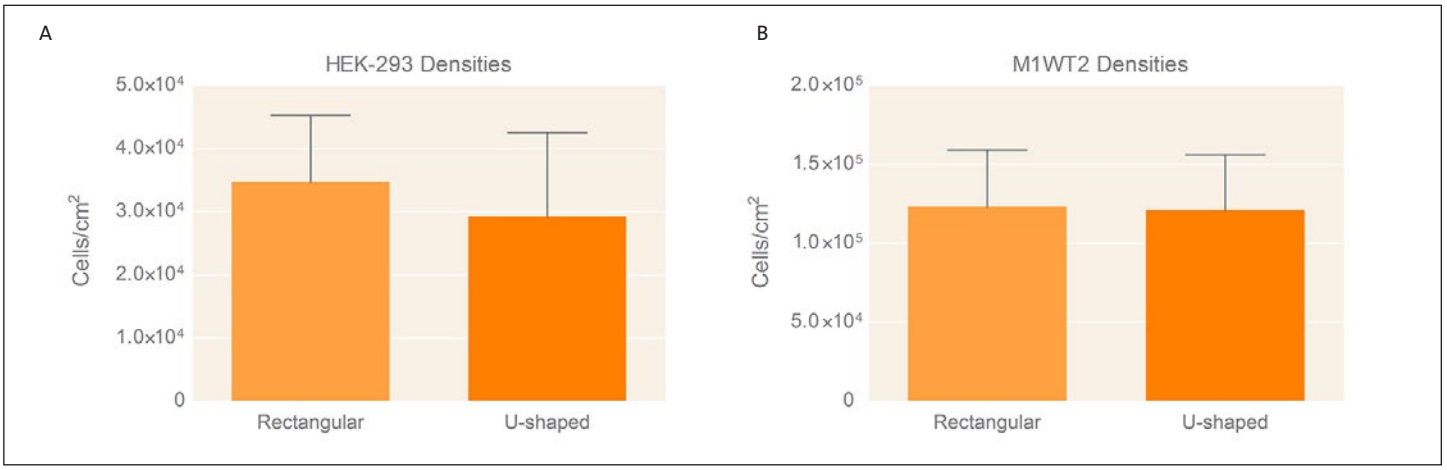

Figure 2. Similar cell growth was obtained under reduced conditions. (A) HEK-293 and (B) M1WT2 cells were expanded, harvested, and enumerated. No statistical difference was observed between both vessels. Paired T-test. n = 9.

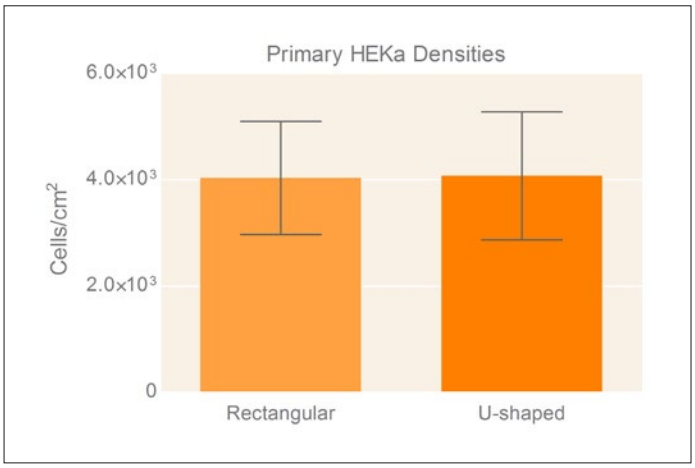

Figure 3. S Similar cell growth was obtained with a primary cell line. Primary HEKa cells were expanded for 4 days, harvested, and enumerated. No statistical difference was observed between both vessels. Paired T-test. n = 9.

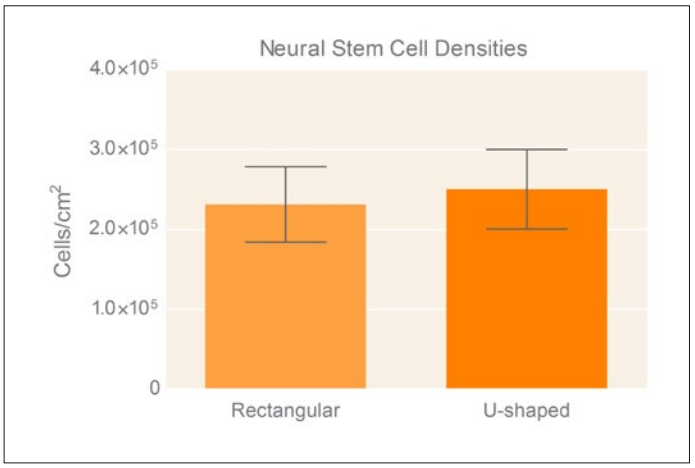

Figure 4. Similar neural stem cell densities were obtained after flasks were exposed to various temperatures. Flasks were pre-coated with poly-L-ornithine/laminin. Neural stem cells were expanded for 4 days, harvested, and enumerated. No statistical difference was observed between both vessels. Paired T-test. n = 9.

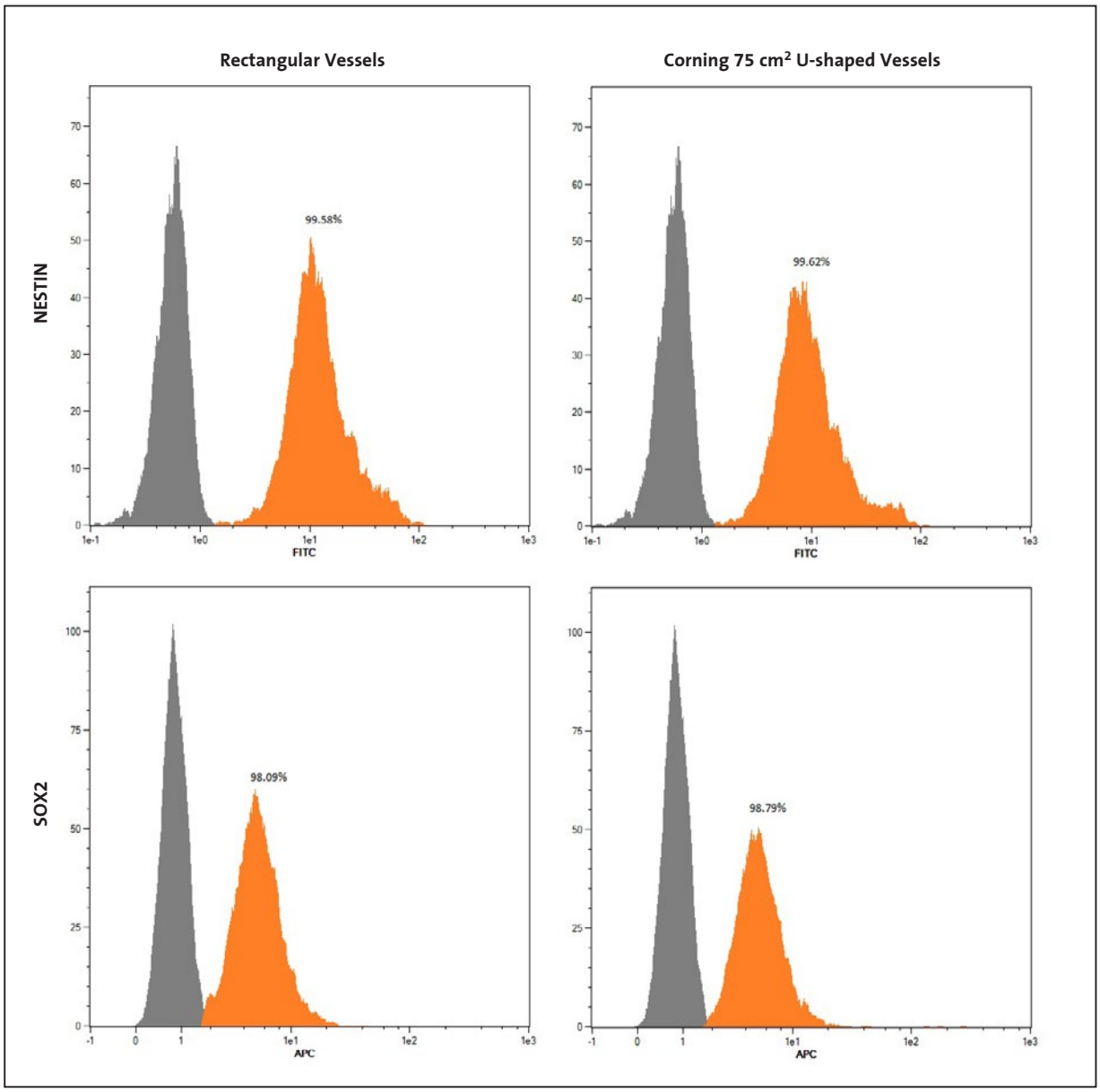

Figure 5. Neural stem cells cultured on the Corning® 75 cm^2 U-shaped flask retained multipotency. Representative histograms of nestin (top) expression and SOX2 (bottom) compared to isotype controls (gray) from NSCs harvested from both rectangular and U-shaped vessels.

Conclusions

- There was no statistical difference in HEK-293 and CHO-K1 cell growth between the rectangular and U-shaped vessels.
- There was no statistical difference in cell growth or adherence between the two designs with a reduced serum concentration.
- Variations in temperature (4°C to 37°C) during the coating procedure did not compromise the integrity of the vessel.
- Neural stem cells expanded similarly with no impact on multipotency.
- Primary HEKa cells expanded in U-shaped flasks as expected and achieved similar densities to those cultured in traditional rectangular flasks.

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