LTI (NY/USA) Summary Report: Evaluation of Res-Del Solutions

Res-Del RS-I and RS-E Solutions were thoroughly evaluated in embryonic stem (ES) cell culture for their potential in supporting cell attachment and growth, and as a holding media.

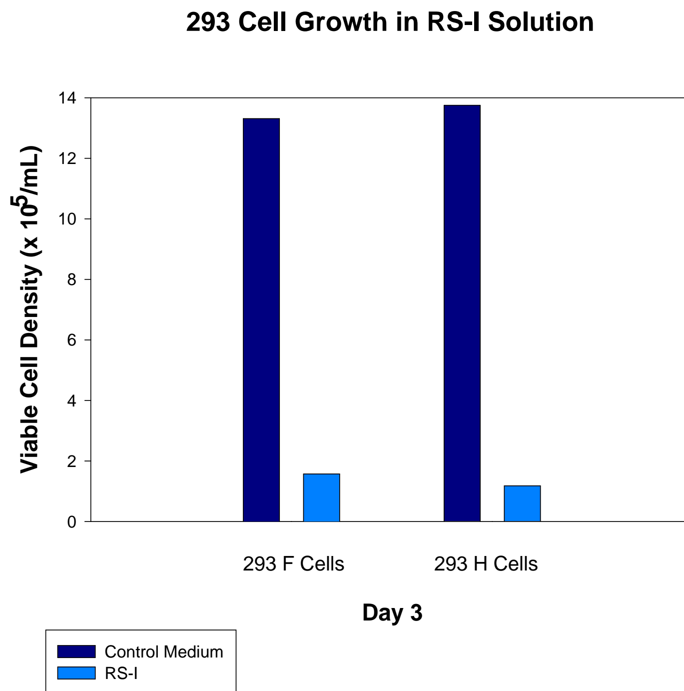
ES Cell Attachment and Growth

D3 ES cells were plated in RS-I Solution or RS-E Solution at low density, +/- ESGRO, on inactivated murine embryonic fibroblasts (feeder cells) or on gelatin-coated plates. Cultures were observed on days 3, 5 and 7 post-plating, and observations were recorded. Neither RS-I nor RS-E Solution supported adequate ES cell attachment on the feeder cells, nor on the gelatin-coated plates, at either cell density. Furthermore, feeder cell morphology had greatly declined. ESGRO supplementation had no effect on the cultures (it normally enhances cell attachment and prevents ES cell differentiation). In another study, RS-I and RS-E Solutions were supplemented with 15% KNOCKOUT™ SR, and evaluated on ES cells co-cultured with feeders, +/- ESGRO. The addition of the KNOCKOUT™ SR enabled *some* ES cell attachment, but growth was very poor as compared to the controls.

Holding Media

Both RS-I and RS-E Solutions were evaluated as holding media for ES cells, using a KNOCKOUT™ SR control. ES cells maintained a higher viability in the KNOCKOUT™ control over a three-day period (see Figure 1).

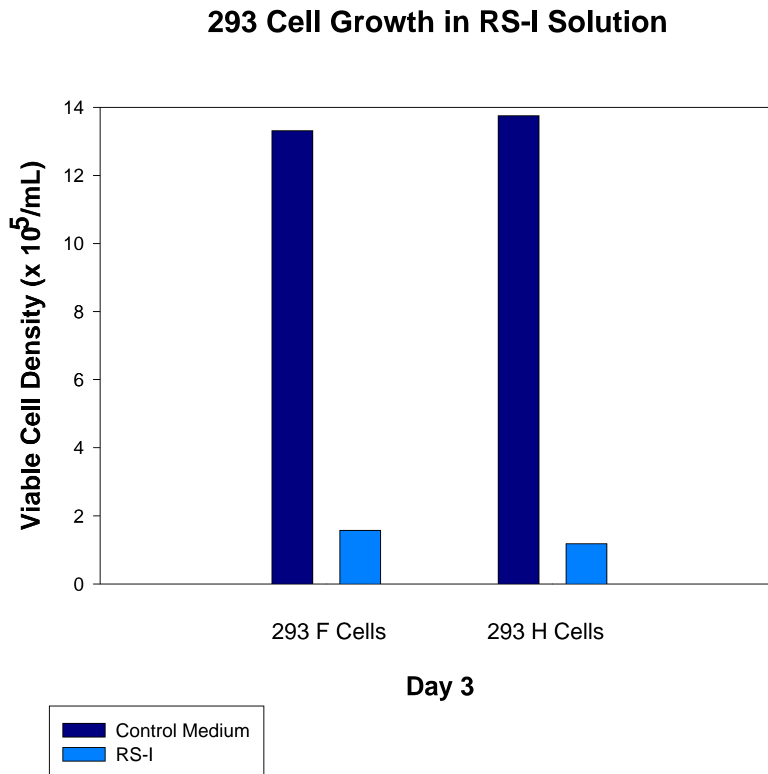
Figure 1



293 F and 293 H Cell Growth

RS-I Solution was also evaluated for the growth of two different 293 cell lines (human embryonic kidney) in suspension culture. 293 F and H cells were taken from serum-free medium and directly plated in RS-I Solution. Viable cell counts were taken three days post-plating (see Figure 2). The RS-I cultures were 32% and 57% viable respectively, with no cell growth.

Figure 2



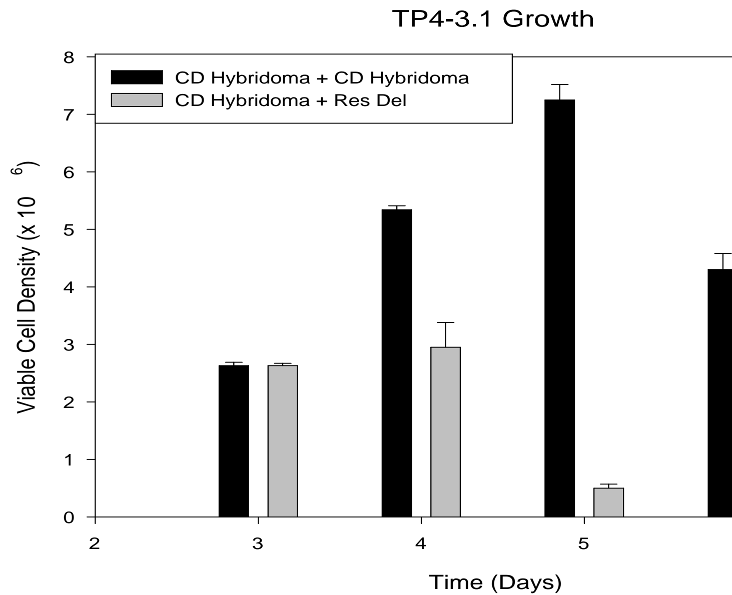
RS-I was used to complex both DNA (pCMVbgal) and cationic lipids, prior to transfection of either ES or 293 cells. DNA and lipids were also complexed in Opti-MEM I and D-MEM as controls. At 24 hours post-transfection, cells were fixed and stained for gal activity. In multiple trials, the controls yielded the highest transfection efficiencies (see table below).

Complex Medium	ES Cells	293 F	293 H
Opti-MEM I	+++	++++	++++
D-MEM	+++	++++	++++
RS-I	++	++	+

+	poor
++	fair
+++	good
++++	excellent

Hybridoma Growth and Productivity

Res-Del solution did not promote monoclonal antibody production by TP4-3.1 cells. (Cells were seeded at 1 x 10E5/ml) in CD Hybridoma Medium and cultured for 3 days. Cultures were centrifuged and resuspended in either fresh CD Hybridoma Medium or Res-Del solution and incubated for 3 more days with viable cell density and IgG monitored daily).



Holding/ Expansion Media for Human Normal Bone Marrow

The purpose of this study was to determine if the Res-Del media formulation presented any advantages over conventional serum supplemented or serum free formulations as a holding medium for the temporary storage of freshly harvested human bone marrow cells.

Experimental Protocol

Normal bone marrow was purchased from Poietic Technologies Inc.

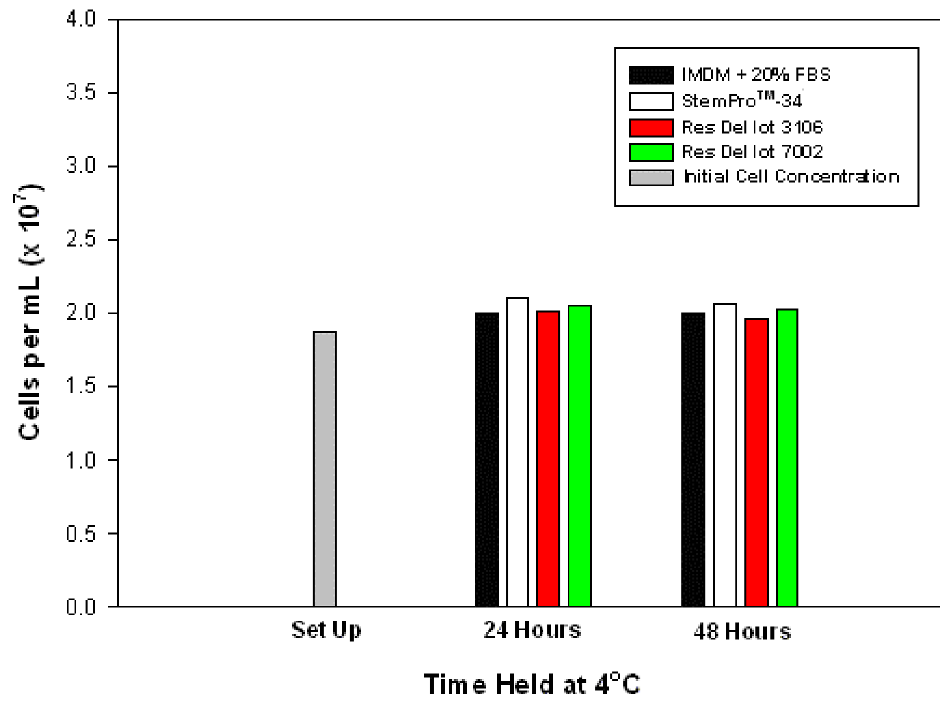
1. At LTI, a cell count of the marrow was determined using a Coulter counter and viability was determined using Trypan blue exclusion.
2. One mL of bone marrow was mixed with one mL of the following media:
   - IMDM + 20% FBS
   - StemPro™-34 without recombinant growth factors
   - Res-Del media lot 3106
   - Res-Del media lot 7002
3. The samples of media and marrow were placed at 4°C. An aliquot of the marrow (i.e. time sample = 0) were stained with antibodies for flow cytometric analysis at Roswell Park. Additional aliquots of the bone marrow were seeded into StemPro™-34 supplemented with the human recombinant growth factors: Stem Cell Factor (100ng/mL), IL-3 (50ng/mL) and GM-CSF (25ng/mL). The cells were incubated for six days at 37°C in a humidified atmosphere of 5% CO_2 and air.
4. After storage at 4°C for 24 and 48 hours, aliquots of the bone marrow in the indicated media were taken for cell counts and viability as described in 3 above. Aliquots were also stained for flow cytometric analysis and *ex vivo* cell expansion as described.

Results

Cell Viability and Cell Counts During Storage - During the time course of this study, cell viability as determined by Trypan Blue dye exclusion, remained essentially 100% for the bone marrow cells, irrespective of the media formulation. During storage at 4°C there was a slight but not significant increase in cell number in all of the formulations tested. These data are shown in Figure 3.

Figure 3

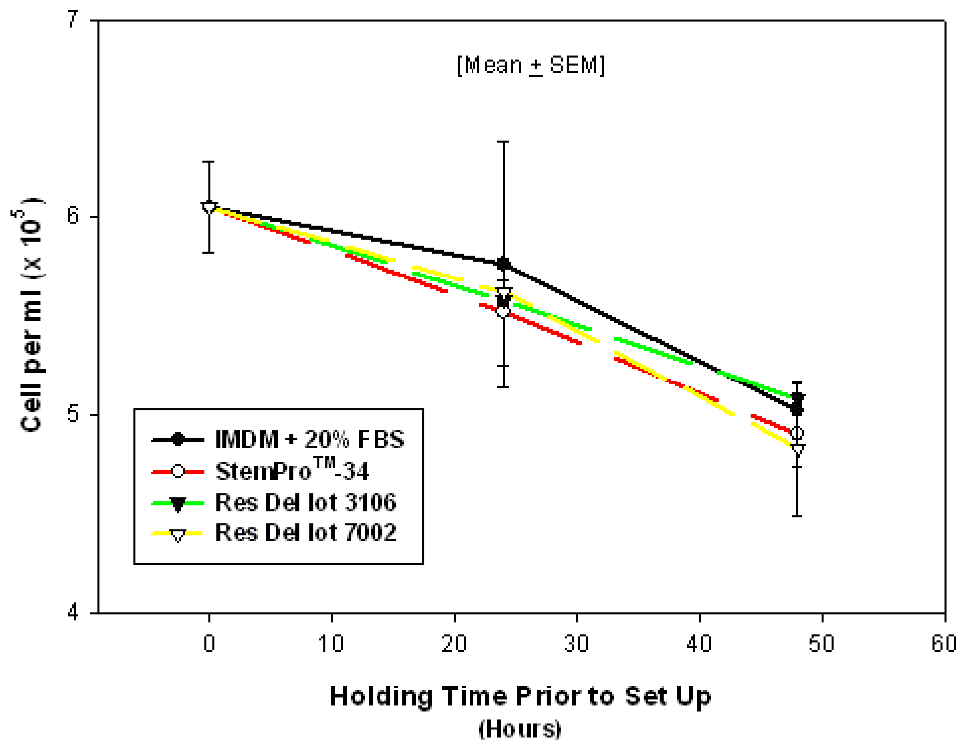
The Effect of Media Formulation and Holding Time at 4°C on Cell Number and Viabilty of Normal Bone Marrow Cells



Ex Vivo Expansion of Stored Bone Marrow - We measured the proliferative potential of the progenitor cells in the bone marrow by culturing aliquots of the marrow in StemPro™-34 supplemented with a combination of human recombinant growth factors demonstrated to promote cell expansion. Aliquots of the bone marrow cells stored in the various media formulation tested were cultured in StemPro-34 supplemented with the human recombinant growth factors SCF (100ng/mL), IL-3 (50ng/mL) and GM-CSF (25ng/mL). The cells were grown for 6 days and then cells counts determined using a Coulter Counter. All of the media formulations tested showed a similar decline in the proliferation of the progenitors cells after 24 and 48 hours storage at 4°C. These data are shown in Figure 4.

Figure 4

The Effect of Holding Media and Time on the Ability of Human Bone Marrow Expansion in Culture



Conclusions

The Res-Del formulation does not appear to offer any significant advantages over either classical cell culture media such as IMDM or newer serum-free formulations such as StemPro-34 for the temporary storage of human bone marrow cells.

However, it is to be noted that in both Res Del lot 3106 and lot 7002 RS-I solutions the glutamine levels had declined to < 200 μmol/L because both batches had passed their expiration dates by 1 and 4 years respectively.