

Introduction

Practical culture methods for expansion of human stem cells are needed for Research and Industrial applications. In general, ingredients of known and unknown composition have been used in stem cell cultures based on past studies and practices. However, to harness the substantial potential of stem cells in treating human diseases, products with improved characteristics are needed to support the manufacturing of stem cells for Cell Therapy use. Safety, definition, cost and consistency are key considerations for all such new stem cell products. Based on data mining and cell-based screens, we have designed the RS-Novo™ defined small molecule metabolic enhancer for expansion of human ESC, MSC and HSC with limited differentiation. Cell growth, viability, phenotype and stem cell potential were endpoints of interest in our studies. Our aim was to minimize media components that would "trigger" stem cells into differentiation, apoptosis, and necrosis. We also aimed to minimize components that would introduce inconsistencies, like serum. Lastly, we omitted cytokines as they vary based on end user's cell type and application (growth and differentiation). Here, we cover some RS-Novo™ results with human MSC and ESC and introduce GEM-Novo™ serum-free medium for a more complete culture system for stem cell expansion.

Materials and Methods

Human bone marrow cells were cultured in plates with each medium, and on day 5 non-adherent cells were washed off. Remaining MSC were counted and cultured in α -MEM medium with 10% FBS (control) or in GEM-Novo™ containing 1x RS-Novo™ supplement and 2.5%, 5% or 10% FBS. Cultures were monitored daily for viability and morphology and fed about every other day. MSC's were collected, counted and passaged on d14 and collected and counted on d21. Results from 2 bone marrows are presented here. In each case, each plate was seeded with only a portion of a bone marrow. Total estimated MSC numbers were therefore based on calculating total MSC's that could be harvested, had the entire bone marrow been cultured in that condition.

Undifferentiated hES cells were cultured and various parameters were monitored. Three wells coated with Matrigel™ were used for each condition. H7 uES cell morphology was determined by phase contrast microscopy at every passage. Flow cytometry was performed at passages 3 and 8 to evaluate uES cell phenotype.

GEM-Novo™ serum-free medium consists of qualified, defined ingredients for stem cell culture. In order to provide flexibility to end users' applications, GEM-Novo was designed not to contain cell-specific growth factors for expansion or differentiation. In the case of hES cells, for example, growth factors such as basic fibroblast growth factor (bFGF) may be additionally required for cell expansion.

RS-Novo™ animal-free supplement is defined and non-nutritional, and can be used in conjunction with GEM-Novo for stem cell culture. It consists of small molecules that regulate growth and viability.

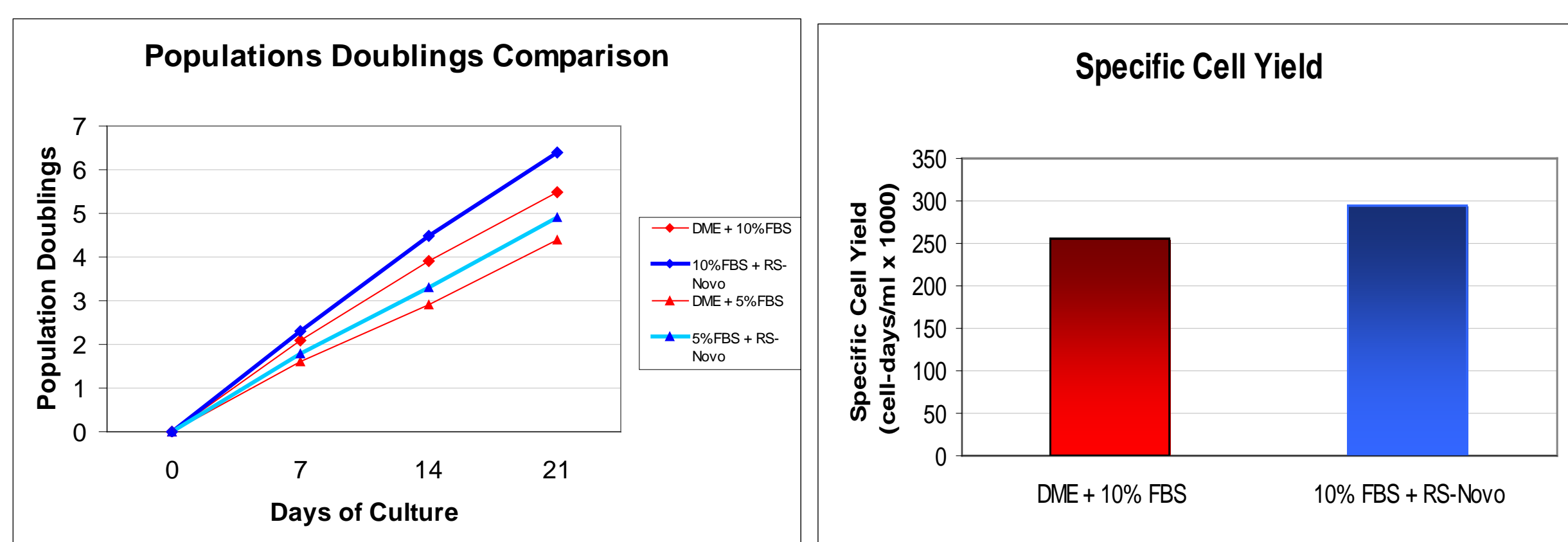


Figure 1: RS-Novo™ increases hMSC yields in serum supplemented basal medium.

A traditional method of growing hMSC has been to use 10% serum. We began here. Even in the presence of serum (5% or 10%) **RS-Novo™** increased cell mass.

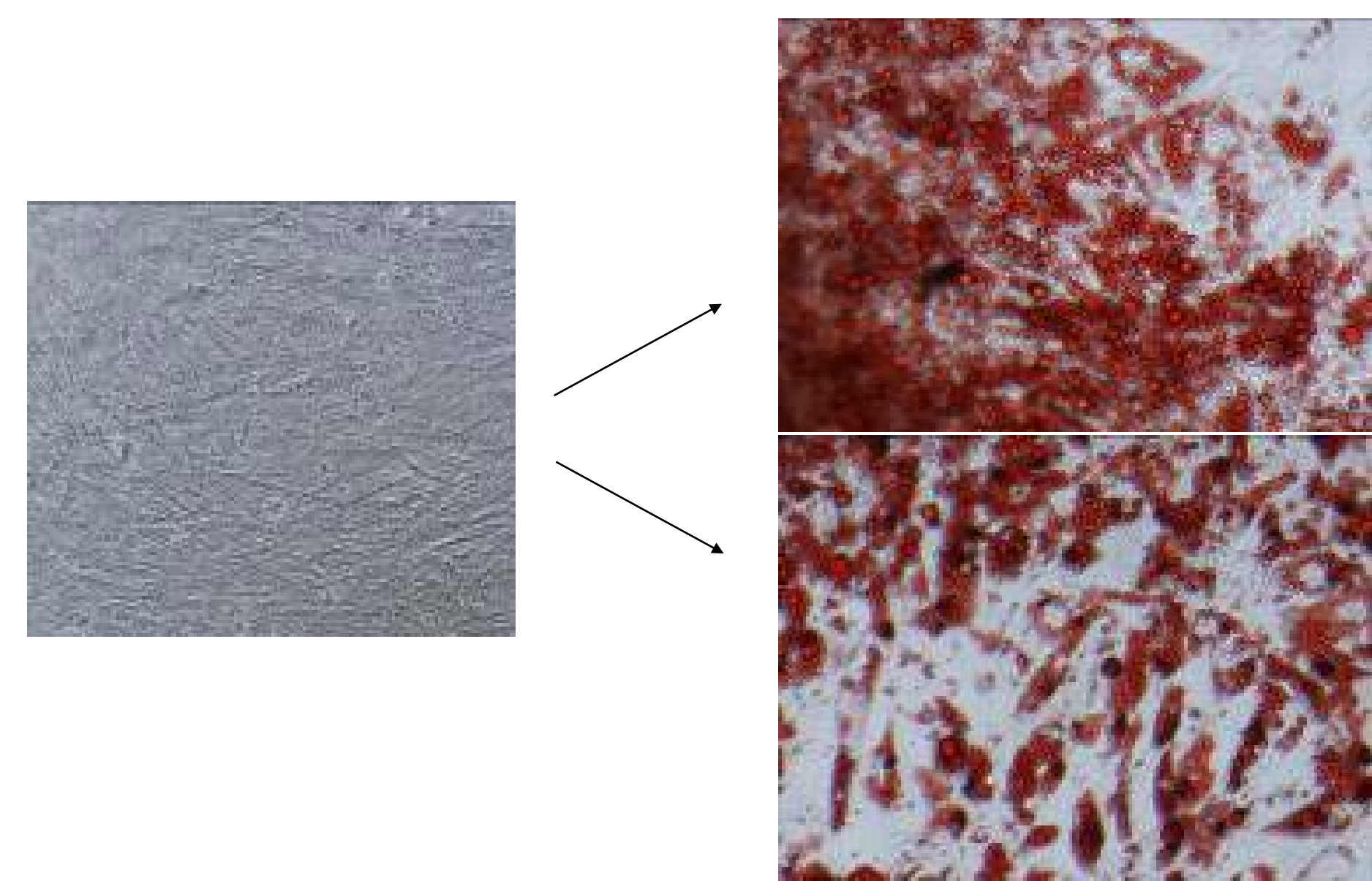


Figure 4: hMSC maintain stem cell potential when grown in GEM-RS.

hMSC's expanded in GEM-RS and subsequently cultured in differentiation media can differentiate into adipocytes. Shown here are Oil Red O staining of adipocytes derived from hMSC expanded in GEM-RS.

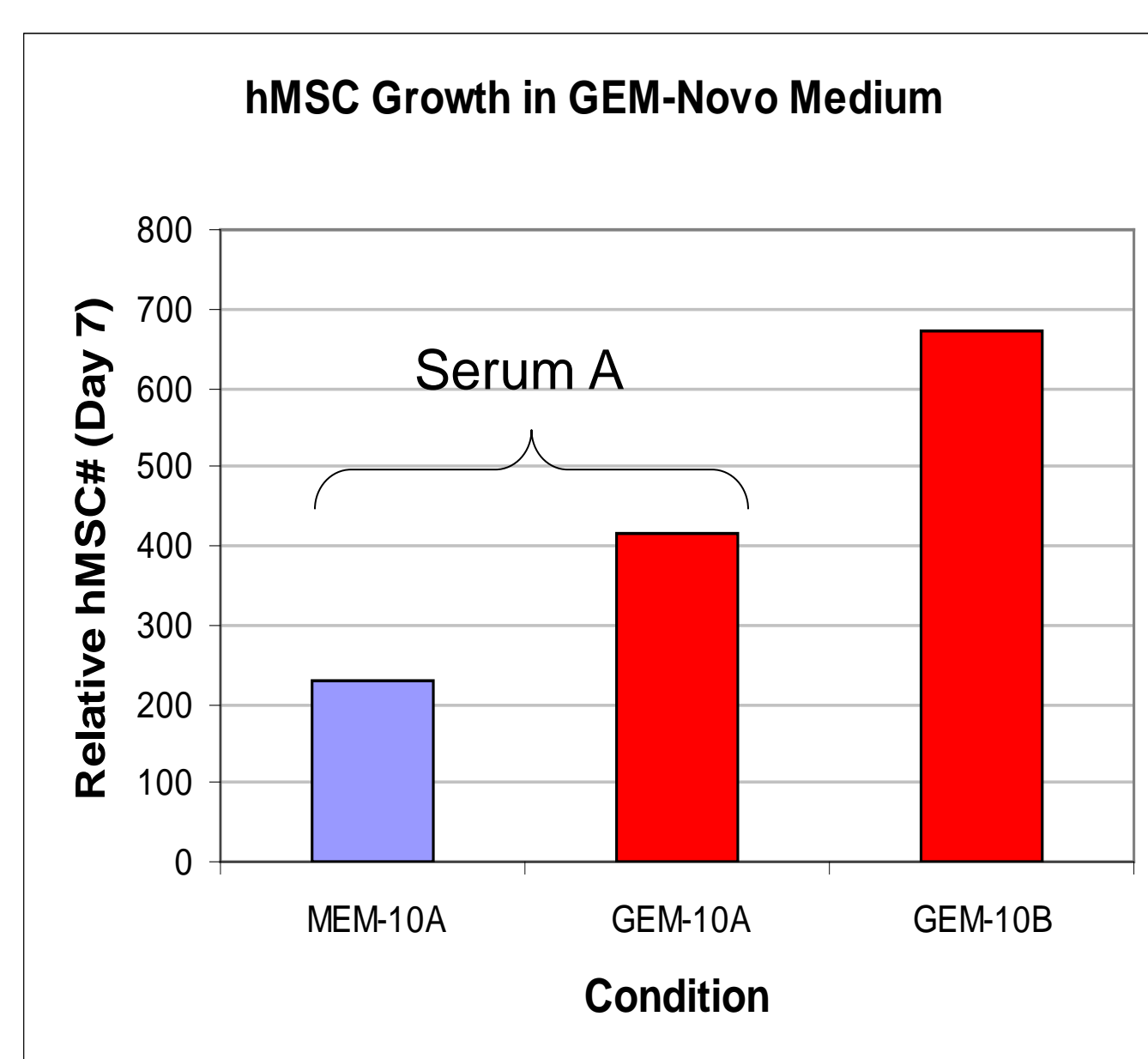


Figure 2: Development of GEM-Novo™ serum-free medium

A more complete cell culture system required that we formulate the new medium **GEM-Novo™**. Like RS-Novo™, GEM-Novo™ was to be compatible with all other additives including serum. As shown here, GEM-Novo™ supported greater cell mass than α MEM, a known benchmark medium for hMSC growth.

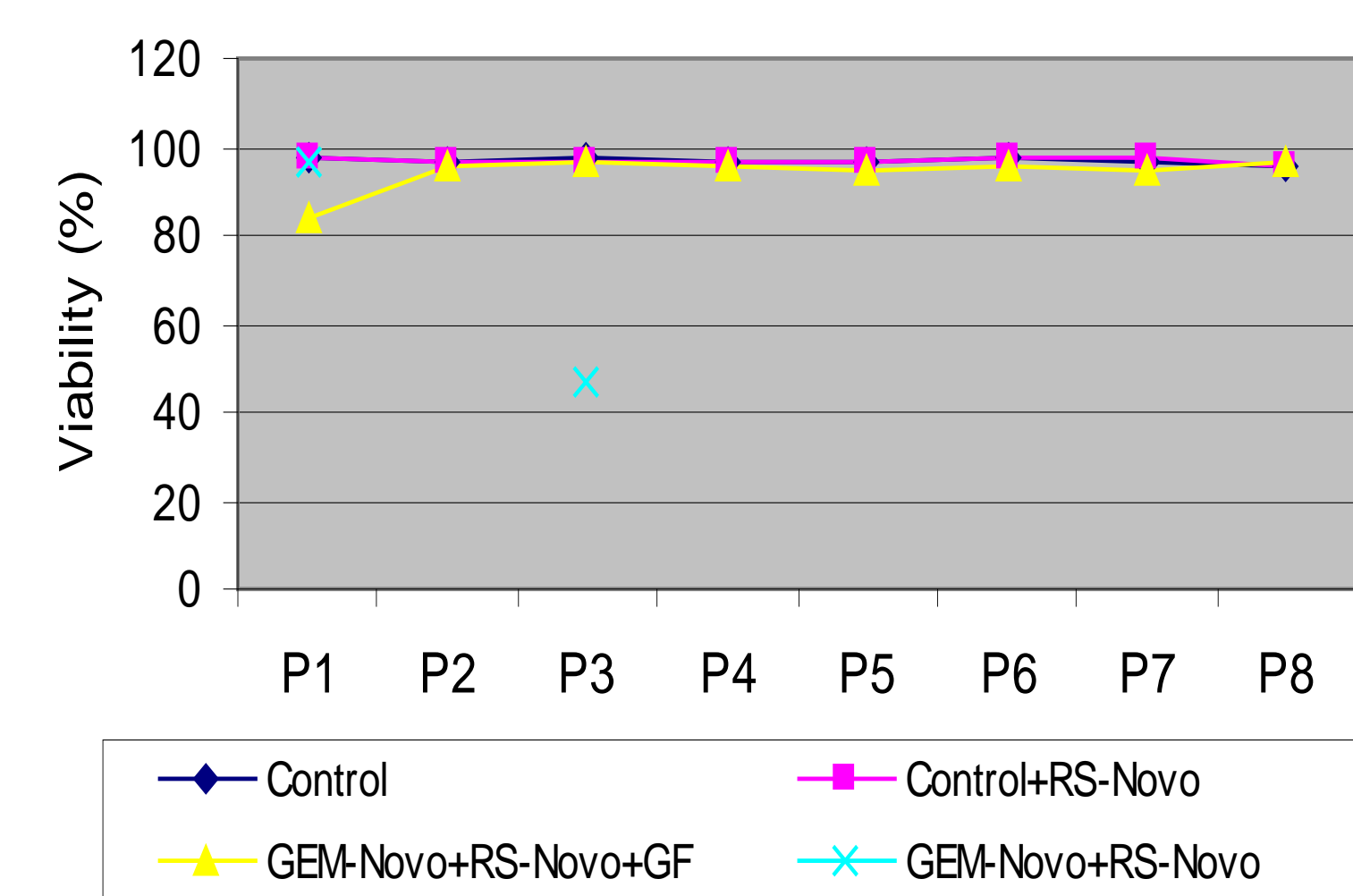


Figure 5: Growth of hESC with GEM-RS.

Human embryonic stem cell H7 clones were cultured in GEM-RS and compared to an optimized condition of an established ESC company. Viability of cells were similar over many passages. Morphologies (not shown) were also similar.

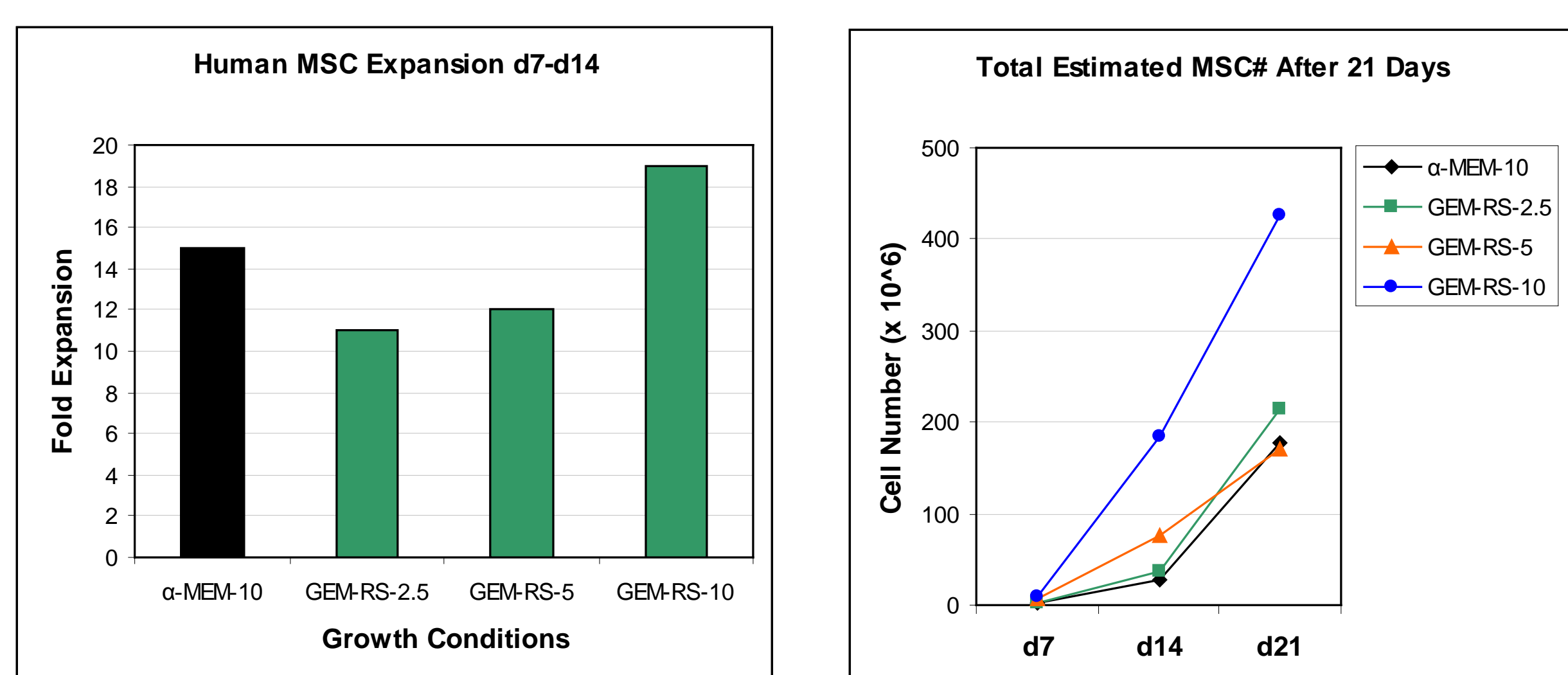


Figure 3: RS-Novo and GEM-Novo increase hMSC yields versus benchmark.

Human MSC from 2 donors were cultured with **GEM-Novo™** and **RS-Novo™** ("GEM-RS"). GEM-RS supported hMSC growth even at reduced serum levels versus α MEM-10.

	Marker	Control Medium	RS-Novo + GEM-Novo Medium
Passage 3	Tra1-60	25.3	38.5
	Oct4	75.8	81.5
Passage 8	Tra1-60	28	36
	Oct4	66.1	81

Table 1. Flow cytometric analysis of hESC markers over 8 passages in culture.

GEM-RS supported consistent marker expression during hESC culture. These data demonstrate that GEM-RS support more consistent undifferentiated hESC phenotype over many passages versus a proprietary in-house medium developed by an ESC company.

Summary

We have presented results with two new products: the RS-Novo™ animal-free supplement and the GEM-Novo™ serum-free medium. Our results indicate that:

- RS-Novo™ supports stem cell growth and limits differentiation.
- RS-Novo™ is compatible with all media tested and their components, including serum.
- RS-Novo™ can be used for culturing human MSC and ESC.
- GEM-Novo™ and RS-Novo™ together afford a consistent, high performance cell culture system for expansion of human stem cells with limited differentiation.