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Introduction

Stem/progenitor cell based therapy is a leading strategy in regenerative medicine as reflected by the large and ever growing number of indexed clinical trials. The prospective use of exogenous endothelial cells to promote tissue repair is gaining attention, particularly in applications of regenerative medicine in which vasculogenesis plays a critical role. Some examples include: large area wound healing, repair of the ischemic heart, vascular transplant and organ recellularization.

To date, media available in the market to grow endothelial progenitor cells or endothelial primary cells are not cGMP compliant or contain xenogeneic elements such as fetal bovine serum (FBS) or even bovine brain extract (BBE). The use of products containing animal serum to grow cells that will be used for clinical applications involves a series of risk factors ranging from immunogenic responses against exogenous antigens to the transmission of diseases.

Here we present the first cGMP, xenogeneic-free and animal serum-free medium for the culture of primary endothelial cells: The culture system EndoGo™ XF Medium (Biological Industries, Cromwell, CT, USA) and PLTGold® (Mill Creek Life Sciences, Rochester, MN).

Results

I. EndoGo™ XF supplemented with PLTMax®

Our original product PLTMax®, derived from normal human donor platelets arose as a xenogenic-free alternative to FBS to be used as a supplement for *in vitro* expansion of human cells used in clinical applications. Currently, there are no products available in the market to grow human endothelial cells that are cGMP and don't use bovine components as a source of protein.

EndoGo™ XF Medium is a cGMP basic medium developed by Biological Industries to grow human endothelial cells. To determine if we could obtain a cGMP complete medium to grow endothelial cells, we supplemented EndoGo™ XF Medium with 5% PLTMax®. This combination was used to grow human umbilical vein endothelial cells (HUVEC) in comparison with the main product available in the market to grow endothelial cells. This competitor medium contains 2% FBS and BBE. HUVEC grew rapidly in the EndoGo™ XF + PLTMax® combination (with an average doubling time of 26h) and demonstrated improved growth rate compared to the competitor medium (Figure 1A and 1C). HUVEC maintained their typical endothelial cell morphology thorough the experiment (Figure 1B). Besides the differences in cell growth between both products, cells grown in the competitor medium went into senescence and started detaching from the plate before reaching confluence (Figure 1B). Cell doublings were consistent across different lots of PLTMax® tested (Figure 2).

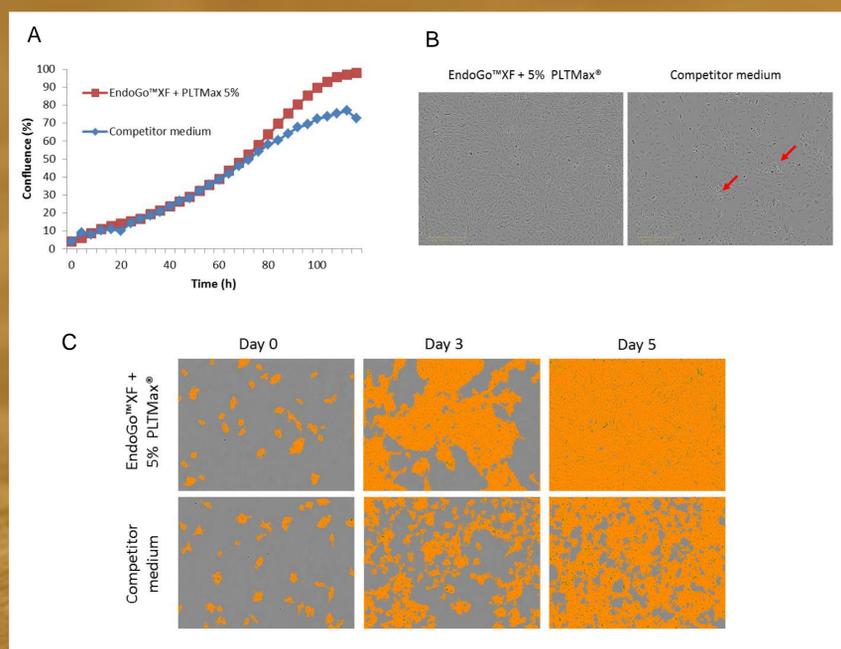


Figure 1. Comparison between EndoGo™ XF + 5% PLTMax® and a competitor medium containing bovine derived supplements. A) Comparison between cell kinetics of HUVEC cultured in EndoGo™ XF + 5% PLTMax® vs competitor medium. The growth is plotted according to the time in culture and the increase in cell density as measured by confluence. B) Appearance of cells grown in EndoGo™ XF + 5% PLTMax® or competitor medium at day 5 of culture. Red arrows point to areas of cell death C) Representative images of HUVEC at day 0, day 3 and day 5 of real time monitoring analysis. Cells are false color masked by monitoring instrument software to measure growth.

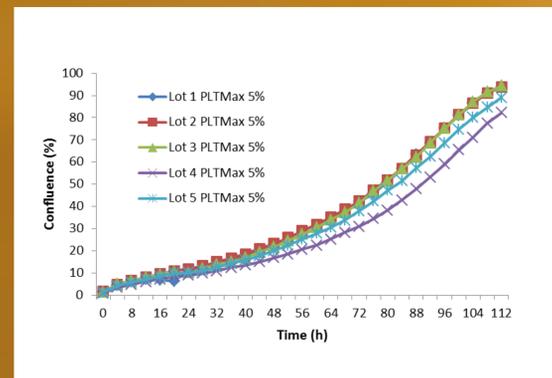


Figure 2. Cell growth consistency for HUVEC expanded in EndoGo™ XF supplemented with different lots of PLTMax®.

II. EndoGo™ XF supplemented with PLTGold®

Due to the presence of certain plasma components such as fibrinogen and coagulation factors, the use of PLTMax® involves the addition of an anticoagulant (heparin) to the cell culture media to prevent clotting. Heparin is purified and refined from swine according to cGMP methods and is one of the most widely prescribed and used drugs in humans. There has been no overt regulatory request for removal of heparin from processes that will be part of a clinical application. However; some quality systems have interpreted existing regulations as a requirement for removal of xenogeneic components.

To eliminate the need for heparin addition, Mill Creek Life Sciences developed PLTGold®, a step forward in the evolution of hPL. PLTGold® is xenogeneic-free, Heparin-free, unfractionated and clot-free.

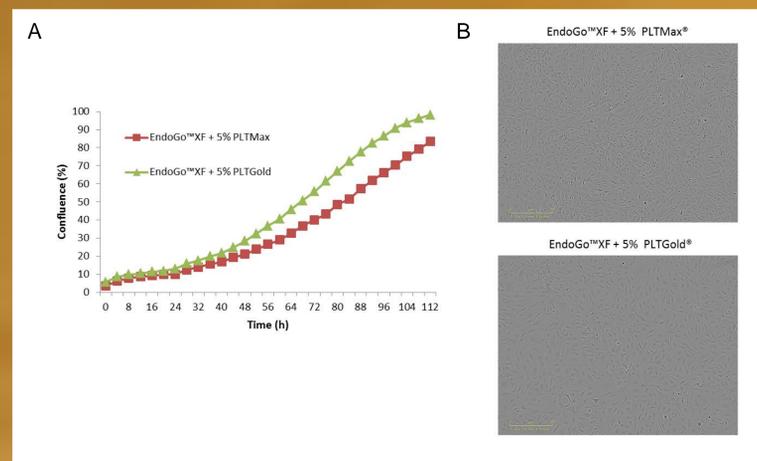


Figure 3. Comparison between EndoGo™ XF + 5% PLTMax® and EndoGo™ XF + 5% PLTGold® to grow human endothelial cells. A) Comparison between cell kinetics of HUVEC cultured in EndoGo™ XF + 5% PLTMax® vs EndoGo™ XF + 5% PLTGold®. B) Appearance of cells grown in EndoGo™ XF + 5% PLTMax® vs EndoGo™ XF + 5% PLTGold® at day 5 of culture.

Growth of HUVEC in EndoGo™ XF + 5% PLTMax® was compared with growth in EndoGo™ XF + 5% PLTGold®. The average cell doubling times obtained for medium supplemented with PLTGold® was 28h compared to the average of 26h obtained for PLTMax® (Figure 3A). However, even when growth was slightly slower in EndoGo™ XF supplemented with PLTGold® compared to the same medium supplemented with PLTMax®, cells continued exponentially growing until reaching 100% confluence without any indication of senescence or cell death (Figure 3B), as opposed to what happened when using the competitor medium containing FBS and BBE (Figure 1B).

Conclusions

- EndoGo™ XF supplemented with PLTMax® is a cGMP and animal serum-free medium that allows the *in vitro* culture of human primary endothelial cells in a faster, healthier and more efficient way than other products available in the market.
- EndoGo™ XF supplemented with PLTGold® promotes endothelial cell growth in a more efficient way than other products available in the market (cells don't reach senescence before reaching confluence) but slightly slower than EndoGo™ XF supplemented with PLTMax®.
- EndoGo™ XF + PLTGold® is a cGMP compliant medium and a completely xenogeneic-free approach that provides an excellent environment to grow human primary endothelial cells, demonstrating improved growth rate compared to other media commercially available.