Development and Characterization of an *In Vitro* Co-culture Angiogenesis Assay System (Angio-***Ready***™; ATCC® ACS-2001-2™) Using hTERT-immortalized Cells for High-throughput Drug Screening

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Abstract

Angiogenesis is a multi-step physiological process which is involved in a large number of normal and disease state processes; In vitro angiogenesis models provide very useful tools to study these processes, one of which is the analysis of tubule formation. Tubules formed in co-culture assays were significantly more heterogeneous and more closely resembled capillaries than Matrigel® tubules. Current co-culture models using primary cells have donor variability, and inconsistent results due to lot to lot variation. In this study, we established an in vitro co-culture model system consisting of an assay ready mixture of an aortic endothelial cell line TeloHAEC-GFP (hTERT immortalized human aortic endothelial cell line) and a hTERT immortalized adipose-derived mesenchymal stem cell line (hTERT-MSCs) in a specially formulated medium containing VEGF supplement (Angio-Ready™ Angiogenesis Assay System). Both cell lines were immortalized by hTERT (human telomerase reverse transcriptase) alone and have been well-characterized showing that the cells retain the most important characteristic of their parental counterparts. The new co-culture system forms functional tubular structures in less than 7 days, and in addition, the hTERT-MSC cells which surround the tubular structures have undergone transformation indicated by elevated positive αSMA staining (a marker of smooth muscle cells), indicating that the system has physiological relevance. Notably, our results showed the co-culture system has minimal lot-to-lot variation indicated by the treatment of three lots with the anti-cancer drug, Ramucirumab (Cyramza®), which also targets the VEGF pathway. Next, we tested the new system with compounds that impact angiogenesis, results demonstrated that the angiogenesis system responds positively to elevated doses of VEGF and negatively to increasing concentrations of suramin; more importantly, the tubular formation efficiency is reduced or blocked by well-known anti-cancer drugs such as Sunitinib (SUTENT®) and Bevacizumab (Avastin®), both of which target the VEGF pathway. Finally, we used the Angio-Ready™ system validated 4 HIF-1(hypoxia inducible factors-1) inhibitors which have anti-angiogenic properties identified by high-throughput screening methods; data showed the results of the new system match with other screening methods including a system screening time as short as 3 days. Therefore, the co-culture model developed by using hTERT-immortalized cell lines described in this report provide a consistent and robust in vitro system for studying cardiovascular biology, drug screening and tissue engineering.

Introduction

- Angiogenesis is a multi-step physiological process; it is also involved in a large number of disease states.
- *In vitro* angiogenesis models provide very useful tools to study angiogenesis; additionally, this model can be used in drug screening applications.
- Tubules formed in co-culture assays were significantly more heterogeneous and more closely resembled capillaries than tubules formed in Matrigel® matrix (Corning)[1].
- A few *in vitro* co-culture models have been developed using primary cells, however, donor variability, low cell quantity per lot, and the short lifespan of primary cells limit their usefulness and consistency.
- In this study, we established an *in vitro* co-culture model system using cell lines that were immortalized by hTERT alone.
- Systematic procedures have been employed to validate this co-culture model using VEGF pathway-related compounds.

Results

1. Angio-*Ready*™ Kit is a "thaw, seed, and go" easy and simple process

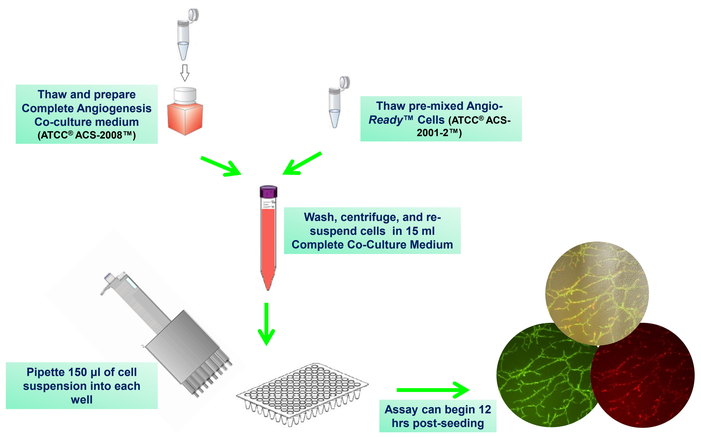


Figure 1. Angio-*Ready*™(ATCC® ACS-2001-2™) Assay overview: "thaw, seed, and assay".

2. TeloHAEC-GFP and hTERT-MSC co-culture model is similar to *in vivo* physiology

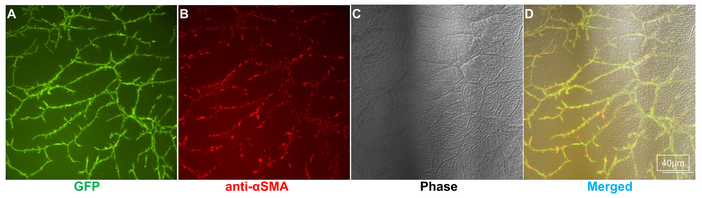
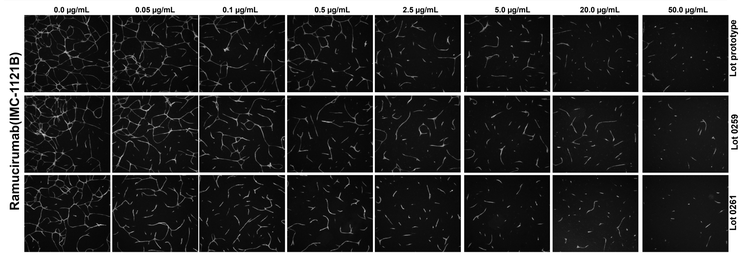


Figure 2. Establishment of TeloHAEC-GFP and hTERT-MSC co-culture angiogenesis. TeloHAEC-GFPs co-cultured with hTERT-MSCs for 7 days in the optimized angiogenesis medium displayed a long branching organization (A) and exhibited immuno-reactivity to an αSMA antibody (Sigma) (B), which co-localized with the TeloHAEC-GFPs (D). Phase contrast microscopy indicated the 3-dimensional structure of the tubes (C).

3. The angio-*ready*™ kit is a robust system for compound screening and is very consistent between multiple different kit lots



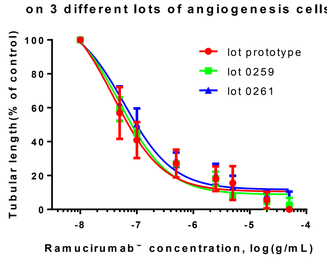
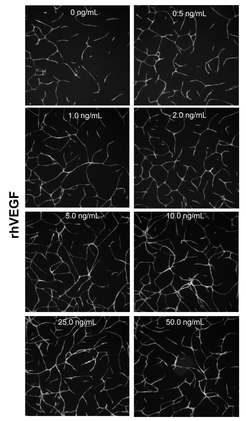


Figure 3. Assay qualities are consistent between different lots of the Angio-*Ready*™ kit. 3 different lots of the Angio-***Ready***™ kit were subjected to angiogenic assays according to manufacturer instructions; 12 hours after the cells were seeded, the system was treated with different doses of ramucirumab (Eli Lilly). Images were taken on day 7 at 4x magnification. Analysis was performed using Array Scan™ XTI and CellInsight™ CX7 (Thermo Fisher Scientific™). (n=3)



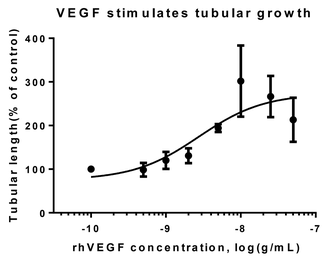


Figure 4. The Angio-*Ready*™ assay system responds positively to VEGF treatment. The Angio-***Ready***™ kit was subjected to angiogenic assays according to manufacturer instructions; 12 hours after the cells were seeded, the system was treated with different doses of rhVEGF. Images were taken on day 7 at 4x magnification. Analysis was performed using Array Scan XTI and CellInsight.(n=3)

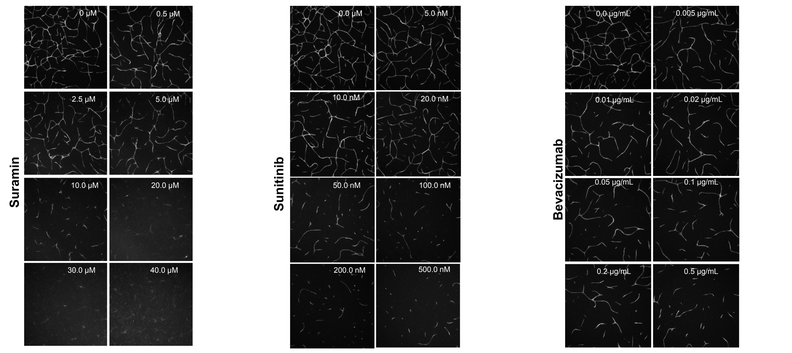


Figure 5. The Angio-*Ready*™ assay system is sensitive to well-known VEGF inhibitors including two clinically-approved prescription cancer drugs. The Angio-***Ready***™ assay was conducted according to manufacturer instructions; 12 hours after cells were seeded, different doses of compounds such as suramin, sunitinib (Pfizer), and bevacizumab (Genentech) were added. Images were taken on day 7 at 4x magnification. Analysis was performed using Array Scan XTI and CellInsight CX7. (n=3)

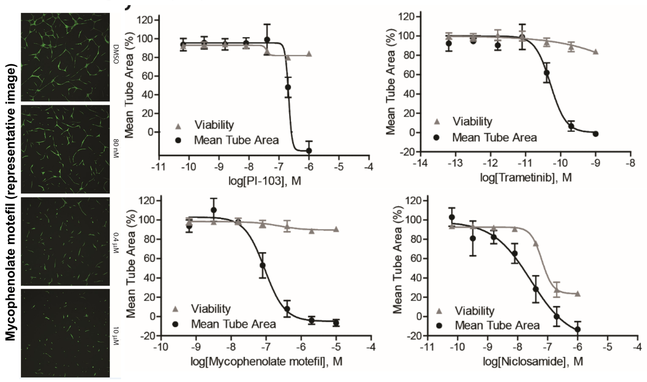


Figure 6. The Angio-*Ready*™ assay system displays a potent response to anti-HIF compounds. The Angio-***Ready***™ assay was conducted according to manufacturer instructions; 12 hours after cells were seeded, 4 anti-HIF compounds that had been identified through other high content screening methods were tested using the Angio-***Ready***™ system. Images were taken on day 3 at 4x magnification. Analysis was performed using Array Scan XTI. (n=3)

Summary

- Fine tubular structures formed in less than 7 days in the TeloHAEC-GFP/hTERT-MSC co-culture system, and the MSCs, which surrounded the tubular structures in an *in vivo*-like manner, expressed a marker of smooth muscle cells.
- Tubular formation efficiency is increased by VEGF stimulation and decreased by suramin, sunitinib, and bevacizumabin in a dose-dependent manner.
- No lot-to-lot variation is seen between 3 different lots of the Angio-***Ready***™ system when treated with ramucirumab.
- Test compounds identified through other external high-content screening methods as being anti-angiogenic block tubule formation in the Angio-***Ready***™ assay, and the assay time can be as short as 3 days.
- This co-culture model provides a consistent and robust *in vitro* system for studying vascular biology and tissue engineering, as well as for high-throughput compound screening.

References

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