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A Long Term Culture Model for Primary Hepatocytes from Cynomolgus Monkeys

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ABSTRACT

In vitro models of animal liver tissues are of great interest to drug developers and regulatory agencies because of their potential to reduce costs and live animal use during drug development. Furthermore, drug metabolism and toxicity studies *in vitro* across human and animal species are utilized for selection of the appropriate animal species for *in vivo* testing in rodent and non-rodent species. Primary monkey hepatocytes are widely considered to be the most suitable for drug metabolism and toxicity studies; however, these cells display a precipitous decline in phenotypic function when kept in suspension or plated in a sandwich of extracellular matrix. We have developed a microscale model of the Cynomolgus monkey liver in which microfabrication tools adapted from the semiconductor industry were utilized to organize primary hepatocytes into colonies of empirically optimized dimensions and these colonies were subsequently surrounded by supportive murine embryonic 3T3-J2 fibroblasts (cynomolgus monkey micropatterned co-cultures or CM-MPCC). We have determined the effects of hepatocyte cell seeding densities, donor lots, and fresh versus cryopreserved sources on the magnitude and lifetime of CM-MPCCs. Several liver-specific functions were measured over four weeks including urea synthesis, cytochrome P450 activities, and formation of functional bile canaliculi. Our results indicate that CM-MPCCs display higher levels of measured functions for at least 4 weeks as compared to traditional cell culture models. For instance, bile canalicular transport was observed with 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate for at least 24 days in culture, while urea synthesis was nearly 45-fold higher in CM-MPCCs than in sandwich cultures on Day 10 of culture (266.87 µg/day/million cells vs. 5.99 µg/day/million cells). In addition, cultures showed higher functionality when proliferating fibroblasts were used as compared to growth-arrested ones. The utility of CM-MPCCs for drug metabolism and toxicity studies is now currently under investigation. In the future, CM-MPCCs may be used as an *in vitro* predictive tool for refining and reducing testing in live monkeys during drug development.

INTRODUCTION

The liver is the primary site of metabolism for xenobiotics. Therefore, drug developers carefully study the effects of new drug candidates and their metabolites on the liver. Pre-clinically, they accomplish this by leveraging various *in vitro* models such as microsomes and primary hepatocytes in monolayer or sandwich cultures, and *in vivo* studies using animals such as rats, dogs, and monkeys. With the increasing costs of drug development[1], better predictive high- and medium-throughput *in vitro* models are necessary to reduce money and time spent bringing candidate drugs to market. Previous work[2] has shown that micropatterning and co-culturing with stromal cells increases the function and longevity of primary human hepatocytes *in vitro*, allowing for more in-depth and physiologically relevant studies. Specifically, human micropatterned co-cultures (h-MPCCs) maintain high enzymatic activity, phase II activity, and liver specific functions for several weeks longer than conventional hepatocyte culture models. The *Macaca fascicularis* or cynomolgus monkey is a non-human primate often used in pre-clinical animal studies. In this study, we applied microtechnology and tissue engineering techniques to cynomolgus monkey hepatocytes in order to determine if these cells could be stabilized in micropatterned co-cultures similar to their human and rat counterparts.

RESULTS

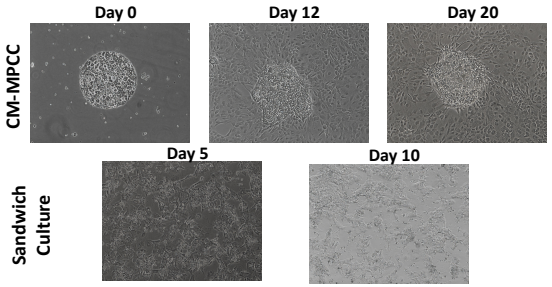
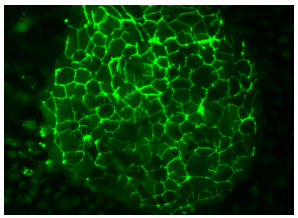
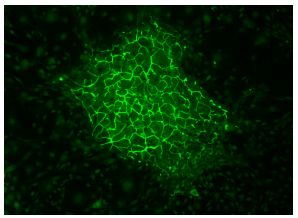


Figure 1. Phase contrast images of pure hepatocytes on day 0 (left) and in CM-MPCC or sandwich culture on various days of culture. Confluence of hepatocyte islands and overall *in vivo*-like hepatic morphology is better maintained for up to 4 weeks in CM-MPCCs as compared to loss of monolayer integrity and cell loss in sandwich cultures.

CRYOPRESERVED FRESH

Figure 2. Bile canalicular transport of 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate on day 28 of culture. Images are of CM-MPCC seeded in 24-well format with either a cryopreserved all-male cynomolgus monkey lot (left, 200X magnification) or freshly-isolated cynomolgus hepatocytes (right, 100X magnification). Overall, similar bile canalicular network forms in islands seeded with either cryopreserved or freshly isolated hepatocytes.





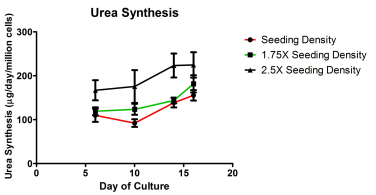


Figure 3. Urea synthesis in CM-MPCC seeded in 24-well format with a cryopreserved lot of pooled male and female cynomolgus hepatocytes.

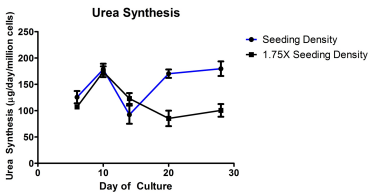


Figure 4. Urea synthesis in CM-MPCC seeded in 24-well format with freshly isolated cynomolgus hepatocytes. Overall, the magnitude and longevity of urea synthesis in cultures created using fresh cells are similar to cultures created using cryopreserved cells.

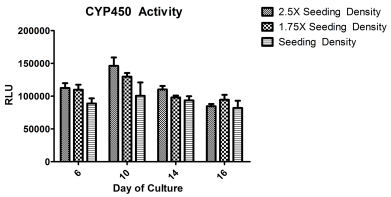


Figure 5. CYP450 activity (as assessed with Promega's Glo assay) in CM-MPCC seeded in 24-well format with a cryopreserved lot of pooled male and female cynomolgus hepatocytes.

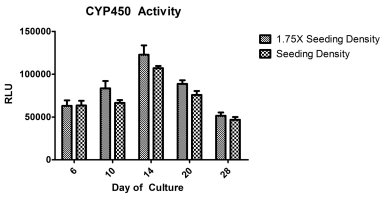


Figure 6. CYP450 activity (as assessed with Promega's Glo assay) in CM-MPCC seeded in 24-well format with a freshly isolated cynomolgus hepatocytes. Overall, the magnitude and longevity of CYP450 activity in cultures created using fresh cells are similar to cultures created using cryopreserved cells.

COMPARISON TO SANDWICH CULTURES

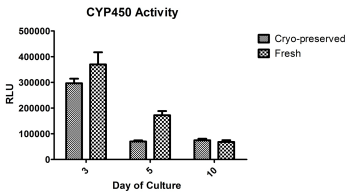


Figure 7. CYP450 activity (as assessed with Promega's Glo assay) over time in sandwich cultures seeded with either cryopreserved male or freshly-isolated cynomolgus monkey hepatocytes. The culture model is unstable regardless of type of cells used, though fresh cells have higher functions out to 5 days as compared to cryopreserved ones.

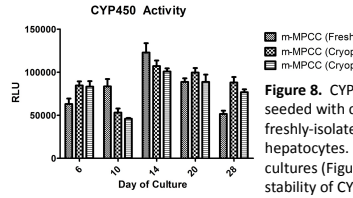


Figure 8. CYP450 activity in CM-MPCCs seeded with cryopreserved male/female or freshly-isolated cynomolgus monkey hepatocytes. In contrast with sandwich cultures (Figure 7), CM-MPCCs show: a) stability of CYP450 activity for at least 4 weeks, and b) minimal functional differences in use of either fresh or cryopreserved hepatocytes.

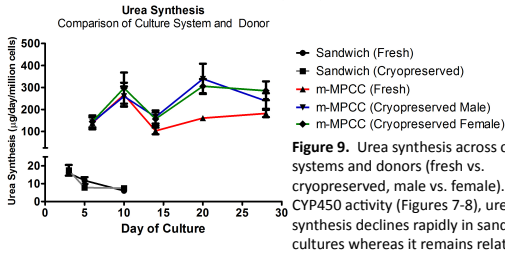


Figure 9. Urea synthesis across culture systems and donors (fresh vs. cryopreserved, male vs. female). As with CYP450 activity (Figures 7-8), urea synthesis declines rapidly in sandwich cultures whereas it remains relatively stable for at least 4 weeks in CM-MPCCs)

CONCLUSION

- The micropatterned co-culture platform (MPCC) is compatible with fresh and cryopreserved, female and male cynomolgus monkey primary hepatocytes. Similar levels and longevity of functions (CYP450 activity, urea synthesis) and morphology are observed with use of various types of cynomolgus monkey hepatocytes.

- CM-MPCCs maintain liver specific functions at higher levels and for several weeks longer (at least 30 days) than sandwich cultures which showed rapid decline over hours to days.

- The MPCC platform can stabilize primary rat, human and cynomolgus monkey hepatocytes and is well suited for applications in drug development, including metabolite identification, clearance predictions, role of transporters (uptake and efflux), drug-drug interactions, and safety assessment.

REFERENCES

1. I. Kola and J. Landis. Can the Pharmaceutical Industry Reduce Attrition Rates? Nat Rev Drug Discov (2004). 3(8): p711-5
2. S.R. Khetani and S.N.Bhatia. Microscale Culture of Human Liver Cells for Drug Development. Nat Biotechnol (2008). 26(1): p120-6