

Quantitative Evaluation of BSEP-Mediated Bile Acid Transport Using an Optimized Hepatocyte Sandwich Model

Abstract # 3546

Minmin Qu, Xin Li, Binlu Huang

MileCell Biotechnology Inc., One Innovation Drive Suite 420, Worcester, MA 01605, USA

Abstract

Sandwich-Cultured Hepatocytes (SCH) provide a physiologically relevant *in vitro* system for investigating hepatobiliary transport and drug metabolism. In this study, we established and optimized an SCH model of primary Sprague-Dawley (SD) rat hepatocytes by refining culture conditions to enhance hepatocyte polarity and canalicular network formation. The model maintained hepatic polarization, functional bile canaliculi, and Phase I/II metabolic activities.

Using this platform, we quantitatively assessed **Bile Salt Export Pump (BSEP)**-mediated bile salt efflux by determining the **Bile salt Excretion Index (BEI)**. Both fresh and cryopreserved primary SD hepatocytes retained canalicular transport function. Classical BSEP inhibitors, Cyclosporine A (CSA) and Ritonavir (RTA), induced a concentration-dependent decrease in BEI. This optimized SCH model provides a robust and reliable *in vitro* system for evaluating hepatobiliary transporter function, predicting drug-induced cholestasis risk, and supporting mechanistic studies in drug safety assessment.

Results

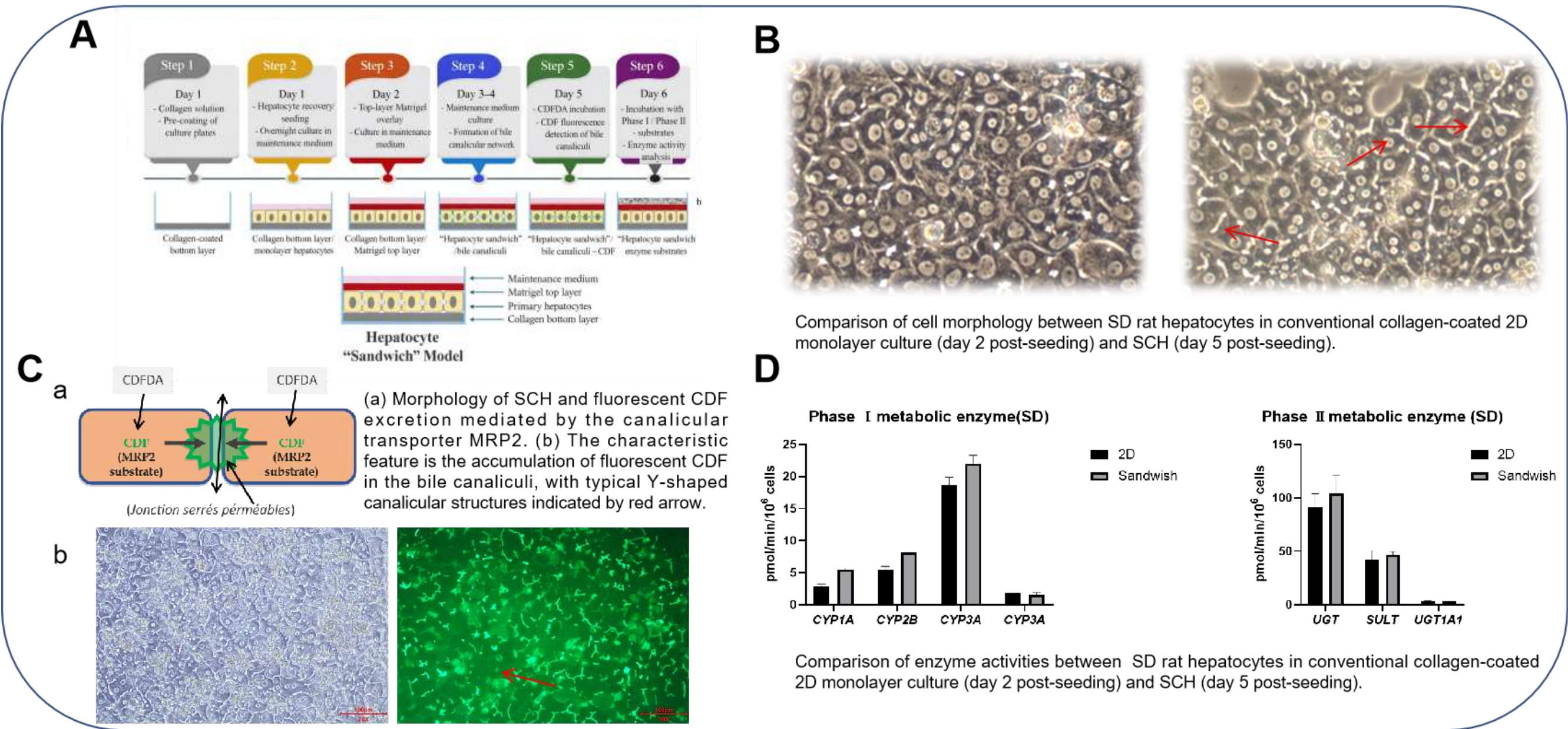


Figure 1: Sandwich-Cultured Hepatocytes (SCH) model using cryopreserved primary Sprague-Dawley (SD) Rat hepatocytes. The SCH model preserves hepatic polarity, canalicular function, and metabolic competence *in vitro*. **(A)** Schematic overview of the SCH workflow, including collagen coating, hepatocyte seeding, Matrigel overlay, bile canaliculi formation, and subsequent functional assays. **(B)** Comparison of conventional 2D culture and SCH of SD rat hepatocytes. Hepatocytes cultured in SCH allow polarization and the formation of intact bile canaliculi, with the canalicular structures indicated by red arrows. **(C)** Principle and results of polarity assessment in SCH. (a): 5(6)-Carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) is cell-permeable and is hydrolyzed by nonspecific intracellular esterases into the fluorescent compound 5(6)-Carboxy-2',7'-dichlorofluorescein (CDF), which is then transported by Multidrug Resistance-Associated Protein 2 (MRP2) and accumulated in the bile canaliculi. This allows the evaluation of MRP2-mediated clearance as well as the formation and distribution of bile canaliculi in SCH. (b): SD rat hepatocytes were seeded at the specified density onto collagen-precoated 24-well plates. Under SCH culture conditions, hepatocytes formed a clear and dense bile canalicular network by days 3-4 of culture (left panel). After incubation with CDFDA for 10-15 minutes, MRP2-mediated CDF fluorescence was observed to be strongly localized and accumulated within the bile canalicular network (right panel). **(D)** Evaluation of metabolic competence. Hepatocytes cultured under SCH (day 5 post-seeding) and conventional 2D monolayer conditions (day 2 post-seeding) were incubated with the specified substrate concentrations at 37°C for 60 minutes. The metabolic activities of phase I and phase II enzymes were calculated based on the formation rates of the metabolites and are expressed as pmol of metabolite formed per minute per 10⁶ cells (pmol/min/10⁶ cells), representing the corresponding enzyme activities. The results indicate that the metabolic activity profiles obtained from the SCH are comparable to those observed under conventional 2D monolayer conditions.

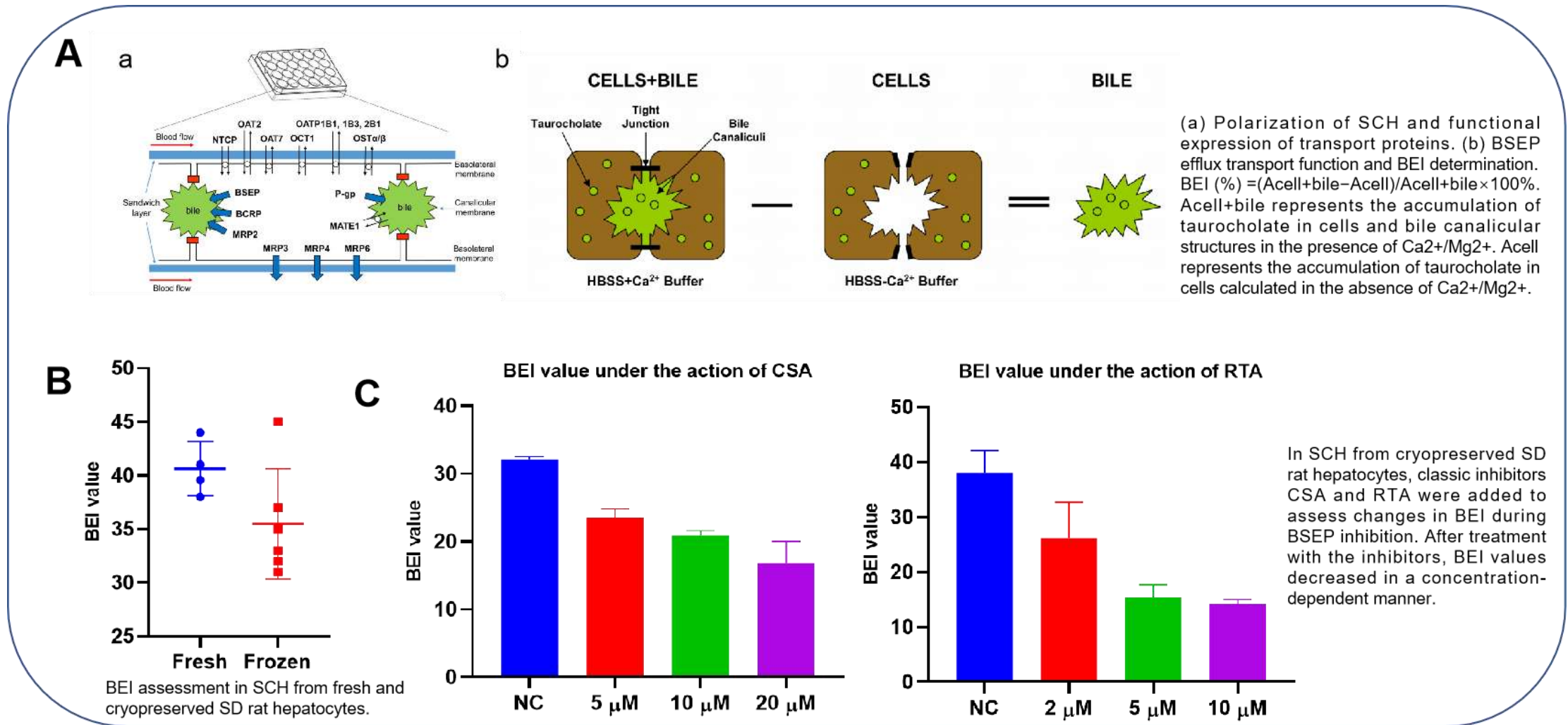


Figure 2: Quantitative Assessment of Bile Salt Export Pump (BSEP)-Mediated Efflux Using the SCH Model. **(A)** Polarity characteristics of SCH allow the formation of functional bile canaliculi, enabling the quantification of BSEP-mediated bile salt transport using the **Bile salt Excretion Index (BEI)**. (a): SCH restore polarity, allowing proper localization of basolateral and canalicular transporters and the formation of a functional bile canalicular network (right panel). This enables the assessment of hepatic uptake and biliary clearance functions through transporters and their substrates. (b): Differences in intracellular bile salt accumulation under standard versus calcium-free conditions reflect canalicular transport activity and inhibitor sensitivity^{1,2}. **(B)** BEI in fresh and cryopreserved SD rat hepatocytes. Fresh hepatocytes exhibited a BEI of approximately 40%, whereas cryopreserved hepatocytes exhibited a BEI of approximately 30%. Although moderately reduced, cryopreserved cells retained functional canalicular transport capacity. Selected batches of MileCell cryopreserved rat hepatocytes have been validated by BEI testing and are available for customer selection. (C) Model validation using BSEP inhibitors. In SCH prepared from cryopreserved SD rat hepatocytes, well-characterized BSEP inhibitors Cyclosporine A (CSA) and Ritonavir (RTA) produced a concentration-dependent reduction in BEI, confirming functional BSEP activity and suitability for transporter inhibition studies.

References

1. Wolf KK et al. *Toxicol In Vitro*. 2010;24:297-309.;
2. Yang K et al. *J Pharm Sci*. 2016;105:443-459.

Conclusion: The optimized SCH model maintains hepatic polarization and functional bile salt transport, enabling reliable *in vitro* assessment of BSEP activity and prediction of cholestasis risk. Contact MileCell team for BEI assay validated lots to reserve the assay-ready cells.