

# Comparison of Metabolic Function Retention Between 2D and 3D Primary Hepatocyte Cultures

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## CONFLICT of INTEREST

All authors are employees and shareholders of MileCell Biotechnology Inc. No other conflicts of interest are declared.

## BACKGROUND and PURPOSE

Primary hepatocytes are widely used to evaluate drug metabolism and metabolic stability. However, 2D cultures may show declining metabolic activity during prolonged culture, whereas 3D systems may better maintain hepatocyte function. This study compared 2D and 3D cynomolgus monkey hepatocyte models using four probe substrates with different metabolic rates: procaine and midazolam (rapidly metabolized) and warfarin and digoxin (slowly metabolized). The objective was to evaluate substrate depletion over 72 h and the persistence of metabolic activity in both culture systems.

## METHODS

Primary cynomolgus monkey hepatocytes were cultured in 2D and 3D systems and incubated with four probe substrates: procaine and midazolam (rapidly metabolized) and warfarin and digoxin (slowly metabolized). Samples were collected at 0, 2, 4, 24, 48, and 72 h. The percentage of parent compound remaining was measured at each time point to characterize metabolic stability and compare the persistence of metabolic activity between 2D and 3D cultures.

## RESULTS

### 1. Metabolic stability of four probe substrates in 2D cell culture

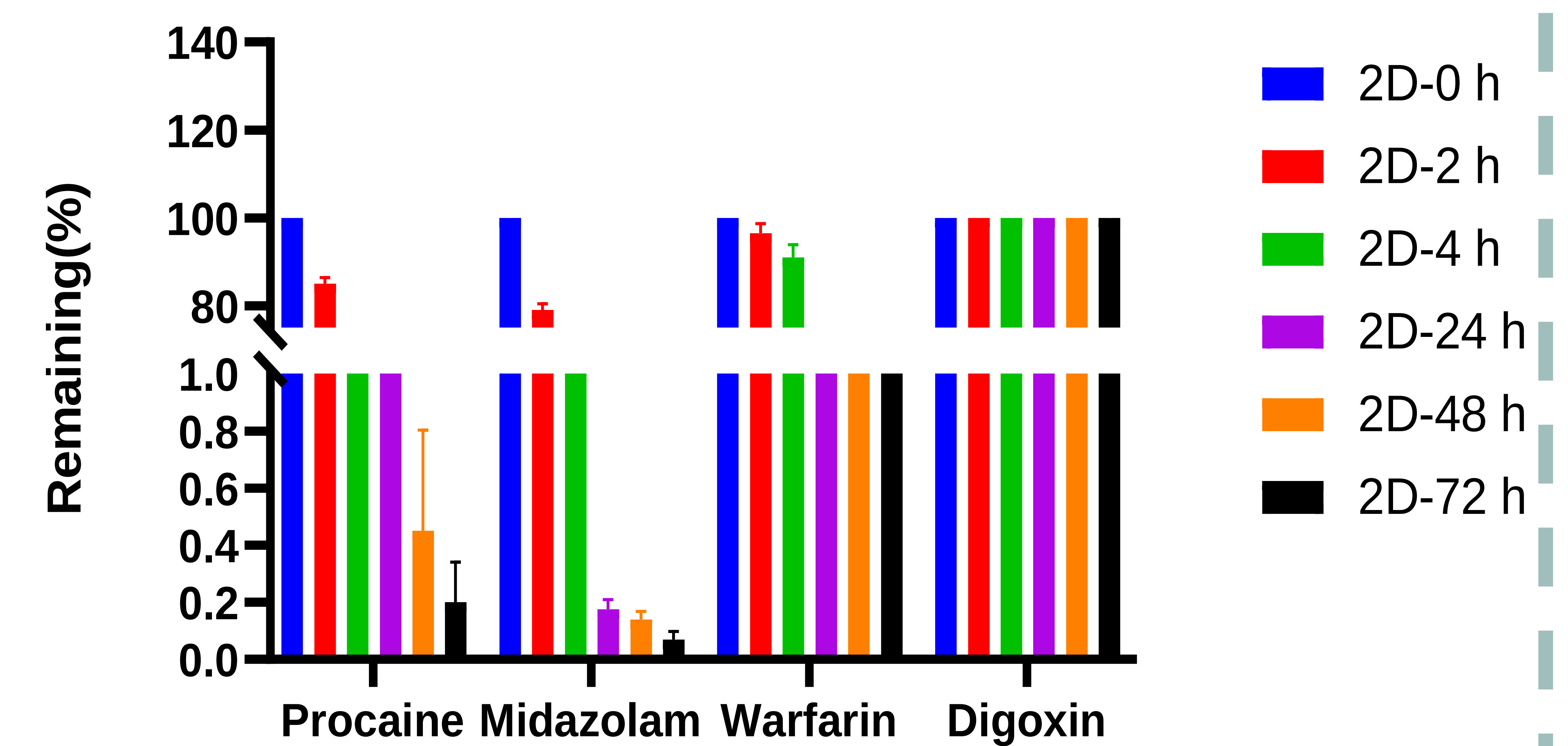


Fig 1. Four probe substrates, including two rapidly metabolized compounds (procaine and midazolam) and two slowly metabolized compounds (warfarin and digoxin), were incubated in a 2D hepatocyte culture system to evaluate substrate depletion and metabolic stability. The remaining percentage of each parent compound was quantified at 0, 2, 4, 24, 48, and 72 h after incubation. As expected, the rapidly metabolized substrates, procaine and midazolam, showed pronounced and progressive depletion during incubation. Procaine decreased rapidly, with only a small fraction of the parent compound remaining at later time points, while midazolam showed substantial depletion as early as 2–4 h and was almost completely depleted by 24 h. These results indicate strong metabolic activity of the 2D hepatocyte model toward rapidly metabolized substrates. In contrast, the slowly metabolized substrates, warfarin and digoxin, exhibited substantially greater metabolic stability. Warfarin showed a gradual decrease in the percentage of parent compound remaining over time, whereas digoxin remained highly stable throughout the 72-h incubation period, with most of the parent compound retained. Data are presented as mean  $\pm$  SD. The y-axis is broken to simultaneously display the relatively high percentage of parent compound remaining at early time points and the very low residual levels observed at later time points for rapidly metabolized substrates.

### 2. Metabolic stability of four probe substrates in 3D cell culture

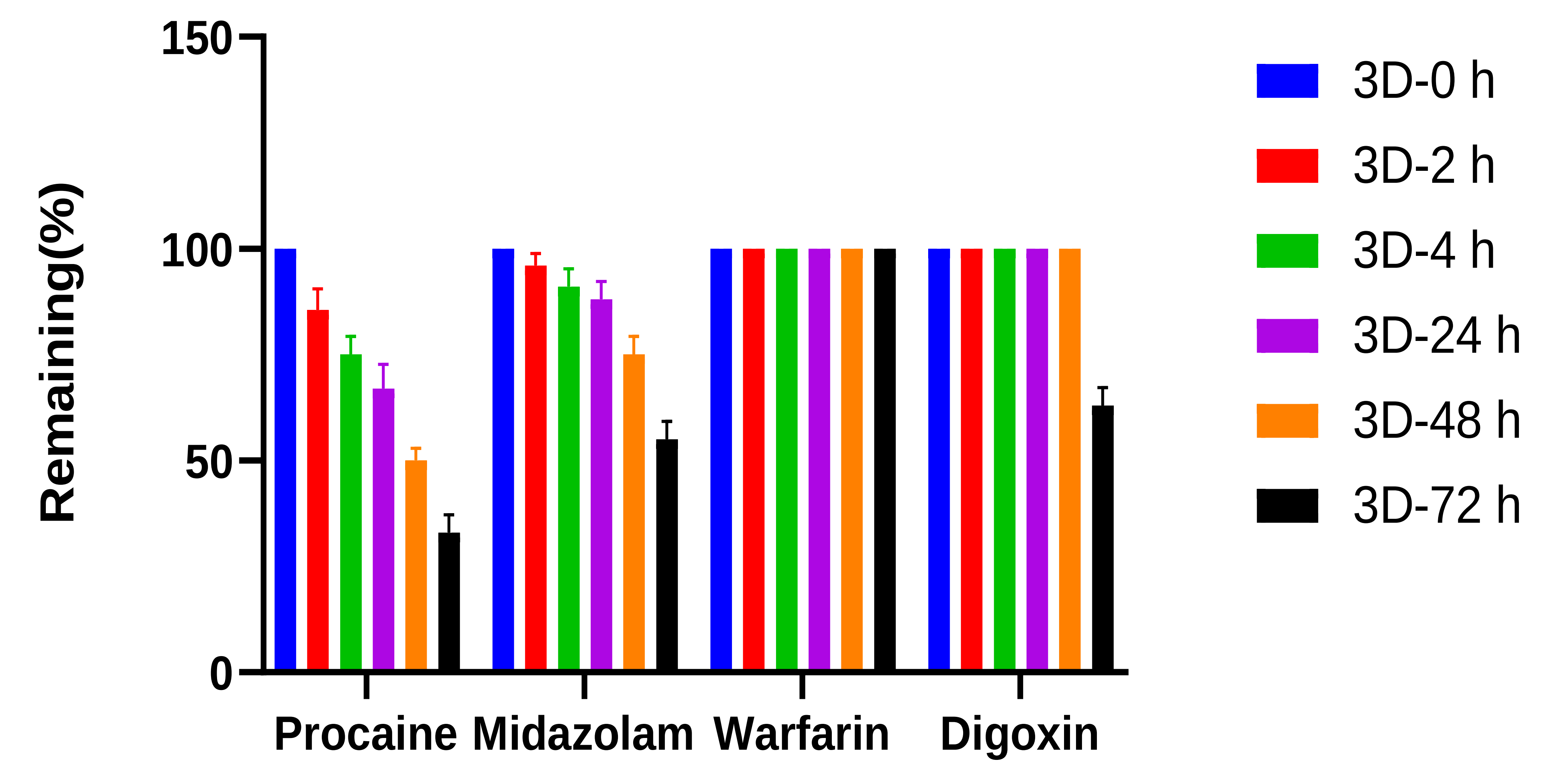


Fig 2. The same four probe substrates, including two rapidly metabolized compounds (procaine and midazolam) and two slowly metabolized compounds (warfarin and digoxin), were incubated in a 3D hepatocyte culture system to evaluate substrate depletion and the persistence of metabolic activity over an extended incubation period. The remaining percentage of each parent compound was quantified at 0, 2, 4, 24, 48, and 72 h. Compared with the conventional 2D monolayer culture, the 3D hepatocyte model showed a more gradual and sustained metabolic clearance profile for the rapidly metabolized substrates. Procaine and midazolam exhibited progressive, time-dependent depletion throughout the 72-h incubation period, without the rapid substrate exhaustion observed in the 2D culture system. For the slowly metabolized substrates, warfarin remained highly stable throughout the incubation period, with nearly 100% of the parent compound retained at all measured time points. Digoxin also demonstrated high metabolic stability, with only a moderate decrease in the remaining parent compound observed at 72 h. Overall, the results demonstrate that the 3D culture system maintained measurable metabolic activity over an extended incubation period while providing a more sustained substrate depletion profile for rapidly metabolized compounds. Data are presented as mean  $\pm$  SD.



## CONCLUSIONS

The 2D and 3D hepatocyte models showed distinct metabolic profiles. Procaine and midazolam showed rapid depletion in 2D culture, while 3D culture demonstrated more gradual and sustained depletion over 72 h. Warfarin and digoxin remained relatively stable in both systems. Overall, the 3D model maintained metabolic activity over an extended period, supporting its potential use for long-term metabolic stability studies.