Rapid Large-Scale Culturing of Microencapsulated Hepatocytes: A Promising Approach for Cell-Based Hepatic Support

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ABSTRACT

Introduction. The efficacy of any bioartificial liver device requires both rapid production and proper bioactivity of the cells for the bioreactor. The goal of this study was to observe the effect of spinner speed and cell density on the proliferation of microencapsulated immortalized human hepatocytes (HepLL) and human hepatoma (HepG2) cells.

Materials and Methods. Alginate-chitosan microcapsulated HepG2 and HepLL cells were randomly divided into 2 groups, and each group was further divided into 8 subgroups according to embedded cell density and spinner speed. The growth, metabolism, and functions of the encapsulated cells in each group were evaluated.

Results. In each group, the cell number, ammonium removal, albumin synthesis, and diazepam clearance increased significantly with the spinner speed, whereas embedded cell density had no impact. Albumin synthesis, removal of ammonium, and diazepam clearance were significantly higher in the microencapsulated HepLL groups than in HepG2 cells at any time point, without any significant difference in cell numbers.

Conclusions. Spinner culture significantly promoted microencapsulated HepLL and HepG2 cell bioactivity. Wrapped cells had optimal function on day 10 in rolling culture groups. These data show that HepLL cells would be a promising candidate for cell-based liver support therapy.

THE EFFICACY of bioartificial liver treatment depends on the bioactivity of the cells in the bioreactor. The minimum quantity of cells required to provide enough liver function has not yet been defined. Assuming that the patient with acute liver failure still has some residual functioning liver mass, a bioartificial liver should contain at least 15% of liver mass. However, given that isolated liver cells in a bioreactor do not have optimal functionality, >15% (preferably 20%–30%) of liver mass is required [1].

Different cell types are used in the bioartificial liver systems, each with its own advantages and limitations [2]. Primary human hepatocytes can be considered, but their availability is limited. Immortalized human hepatocytes (HepLL) derived from normal liver, which retain many liver-specific functions, are currently under study [3–5]. Human tumor cell lines can easily be expanded to large quantities, but concerns regarding their functional capacity in patients limit their applicability. The use of microencapsulated hepatocytes in bioreactor modules is promising for treating patients who suffer from severe liver failure. The encapsulating material has several functions, such as immunosiolation via the exclusion of leukocytes and antibodies, protection of the hepatocyte from shear forces, and an anchorage medium required to maintain functionality [6]. In addition, their large surface area and the ultrathin membrane allow permanent substrates and products to diffuse rapidly.

Alginate is a family of polyanionic copolymers derived from brown sea algae, and it comprises 1,4-linked β-D-mannuronic and α-L-guluronic residues in varying proportions [7,8]. Microcapsules formed by the complex coacervation of alginate and polycations have been extensively used as a scaffolding material for liver cell encapsulation [6].

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analyzed for hepatocyte encapsulation [9,10]. Chitosan is one of the most suitable scaffold materials for liver tissue because its structure resembles that of the glycosaminoglycans [11]. Chitosan is cationic in nature and has high charge density in solution. This allows the formation of stable ionic complexes with multivalent water-soluble anionic polymers under mild physiological conditions [12,13]. The high stability of the alginate-chitosan (AC) complex makes chitosan a better choice for capsule polycation.

The spinner culture of encapsulated cells combines the advantages of encapsulated cell culture and spinner culture. Encapsulated cells are maintained in suspension or attached to small particles, with the advantage of growth space and nutrient solution. Rotation accelerates the exchange of nutrition and metabolites. In addition, cells acquire oxygen more easily, which is conducive to cell metabolism, ensuring rapid growth and the synthesis and secretion of active substances.

Our previous study found that encapsulated HepLL cells, cultured on a large scale in roller bottles, improved cell growth, albumin synthesis, ammonia elimination, and lidocaine clearance compared with free cells cultured in roller bottles [14]. Coward et al [15,16] reported that alginate-encapsulated human hepatoma (HepG2) cells could remain functional in human liver failure. Rotary culture increased their proliferation rates, leading to improved bead packing and a concomitant increase in total protein synthesis, along with maintenance of detoxification capacity. HepG2 cells have become commonly used in many bioartificial liver laboratories [15,17,18]. In the present study, HepLL cells were used as the experimental model and HepG2 cells as a control group. The amplification rate and cell functional differences of microencapsulated HepLL and HepG2 cells in spinner culture were studied to provide a more favorable bioartificial cell selection.

Liver cells are particularly prone to short distance contacts to improve their functions through cell interactions [19]. However, density of the liver cells should also be considered. When it is too high to control cell growth, it reduces cell viability and function. If the density is too low, cell proliferation is too slow, which compromises the clinical requirements. The aim of the present study therefore was to use AC microcapsules of HepLL and HepG2 cells to find a better way to achieve rapid and active growth.

MATERIALS AND METHODS
Chemicals
Dulbecco’s modified Eagle’s medium with high glucose, bovine serum, sodium bicarbonate, penicillin, streptomycin, trypsin-ethylenediaminetetraacetic acid, and trypsin were purchased from Gibco (Grand Island, NY, United States). Alginate (Fluka catalogue no. 71238) and 4-(2-hydroxyethyl) piperazinethanesulfonic acid with 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide or thiazolyl blue (HEPES), 3-(N-morpholino)propanesulfonic acid, calcium chloride, sodium + citrate, and dimethyl sulfosiloxane were purchased from Sigma-Aldrich Co LLC (St Louis, Mo, United States). Chitosan (~85% deacetylated, 30-100 mPas viscosity, water soluble) was purchased from Jinan Haidebei Marine Bioengineering Co, Ltd (Shandong, China).

Table 1. Different Groups According to Cellular Types, Density, and Speed

<table>
<thead>
<tr>
<th>Spinner Speed</th>
<th>HepLL 1.0 \times 10^6 cells/mL</th>
<th>HepG2 1.0 \times 10^6 cells/mL</th>
<th>HepLL 2.0 \times 10^7 cells/mL</th>
<th>HepG2 2.0 \times 10^7 cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 rpm</td>
<td>A1-1</td>
<td>B1-1</td>
<td>A1-1</td>
<td>B1-1</td>
</tr>
<tr>
<td>2 rpm</td>
<td>E1-1</td>
<td>F1-1</td>
<td>E1-1</td>
<td>F1-1</td>
</tr>
<tr>
<td>3 rpm</td>
<td>E2-1</td>
<td>F2-1</td>
<td>E2-1</td>
<td>F2-1</td>
</tr>
<tr>
<td>4 rpm</td>
<td>E3-1</td>
<td>F3-1</td>
<td>E3-1</td>
<td>F3-1</td>
</tr>
</tbody>
</table>

Abbreviations: HepG2, human hepatoma; HepLL, immortalized human hepatocytes.

Cell Culture Groups
The AC-microencapsulated HepLL and HepG2 cells were randomly divided into 2 major groups (HepLL cells in the experimental group and HepG2 cells in the control group). Each major group was divided into 8 subgroups according to embedded cell density (1.0 \times 10^6 cells/mL, 2.0 \times 10^6 cells/mL) and spinner speed (0, 2, 3, and 4 rpm) (Table 1). The initial cell density was 1.0 \times 10^7 cells/mL. Each subgroup was divided into 5 groups for cell proliferative capacity, morphology, hematoxylin and eosin staining and ultrastructural observation (scanning and transmission electron microscopy), albumin synthesis, diazepam clearance, and ammonium concentration assay. Embedded cell-free AC microcapsules were used as the blank control group. They were randomly divided into 4 groups, corresponding to increasing speed (0, 2, 3, and 4 rpm) and treated in the same conditions as the microencapsulated HepLL and HepG2 groups.

Immortalization of Hepatocytes
HepLL, an immortalized human hepatocyte line, was established from a 25-year-old brain-dead male by transfecting it with pcDNA3.1(-) containing simian virus 40 large tumor antigen [19].

Fig. 1. Schematic diagram showing the cell 1-step microencapsulation technique.
Culturing of HepG2 Cells

The basal culture medium used in the present study was Dulbecco’s modified Eagle’s medium with high glucose supplemented with 10% (volume/volume) newborn bovine serum, sodium bicarbonate 3.7 g/L, penicillin 100 U/mL, and streptomycin 100 μg/mL. HepG2 cells were provided by Key Laboratory of Combined Multiple-organ Transplantation, School of Medicine, Zhejiang University. Cell cultures were always initiated in tissue culture flasks and propagated in spinner flasks; the spinners were rotated at a rate of 2 rpm and maintained at 37°C in a 5% carbon dioxide incubator [20].

Entrapment of Hepatocytes

Cell-containing microcapsules were formed by the complex coacervation between polymers by using a method discussed earlier [14,21]. In the jet breakup technique [22,23], a setup consisting of a syringe pump (EMD Millipore Corporation, Billerica, Mass, United States) equipped with syringe and an encapsulator (Nisco, Zurich, Switzerland) was used to form microcapsules (Fig 1). Alginate was autoclaved at 110°C for 20 minutes, mixed with Dulbecco’s modified Eagle’s medium, and stirred overnight to make a 1.5% (weight/volume) solution (pH 7.0), then passed through an 8-μm membrane filter. Chitosan was dissolved in distilled water at a 0.6% (weight/volume) concentration (pH 7.0). Spinner cells were harvested by using trypsin. Hepatocyte viability was >90% based on trypan blue exclusion. The cells were centrifuged before resuspension in alginate solution at a density of 1.0 × 10^6 cells/mL and 2.0 × 10^6 cells/mL; the mixture was pipetted until cell clusters were broken down mostly to single cells. It was transferred into a sterile 50-mL syringe and extruded through a Nisco microencapsulator by using a 200-μm nozzle vibrating at a tunable ultrasound frequency of 25 to 35 kHz. A voltage of 0.57 kV was supplied to the motor pump to maintain the syringe flow at 4 mL/min. The droplets were sprayed into a solution composed of calcium chloride 100 mM, HEPES 13 mM, and 0.5% chitosan. The distance between nozzle and the gelling solution was 14 cm. Under these conditions, spherical calcium-alginate beads with a diameter of about 400 ± 15 μm were formed. After stirring for 30 minutes, all beads were washed in Dulbecco’s modified Eagle’s medium without newborn bovine serum, using a cell mesh, and were all transferred to a spinner (850 cm² surface; Belco Glass, Inc, Vineland, NJ, United States) containing 100 mL of medium. Stirring was initiated immediately at 0, 2, 3, and 4 rpm. Cultures were propagated at 37°C in a humidified incubator containing air with 5% carbon dioxide. The encapsulation procedure was performed under aseptic conditions. The cultures were fed by completely replacing the medium every 48 hours, and medium samples were retained for analysis.

Evaluation of Cell Viability and Number

At 0, 2, 4, 6, 8, 10, 12, 14, and 16 days after entrapped cell culture, the gel beads were initially dissolved in 100 mM of citrate in physiological saline (pH 7.0) at 37°C for 10 minutes with gentle vortexing and centrifuged at 600 rpm for 10 minutes. The total cell concentration and viability were determined by using the trypan blue dye exclusion method and manual cell counting on a hemocytometer.

Determination of Albumin Release

The release of albumin into the culture medium collected every 2 days during the initial 16-day period (on days 2, 4, 6, 8, 10, 12, 14,
and 16) was determined via an enzyme-linked immunoabsorbent assay by using a commercially available kit (Bethyl Laboratories, Montgomery, Tex, United States). Presence of albumin in fetal calf serum did not cross-react with the anti-human ALB antibody used in the enzyme-linked immunoabsorbent assay. The experiment was conducted in triplicate, and the mean value was taken.

Evaluation of Ammonium Clearance

Ammonium clearance was evaluated every 2 days during the initial 16-day period by incubating the hepatocytes with ammonium chloride 1 mmol/L for 2 hours at 37°C before the medium was again replaced with fresh medium. The culture supernatants were assayed for ammonium concentration by using a colorimetric assay (Sigma-Aldrich Co LLC). The experiment was conducted in triplicate, and the mean value was taken.

Diazepam Clearance

Diazepam concentration was determined every 2 days (from days 2–16) by using high-performance liquid chromatography (Waters Corporation, Milford, Mass, United States). A BEH C18 column (Agilent Technologies, Palo Alto, Calif, United States) with an internal diameter of 50 x 2.1 mm and an ultraviolet detector type SPD-10A with a wavelength of 230 nm was used. Diazepam elimination was assessed by incubating the hepatocytes with 5 μg/L of diazepam for 2 hours at 37°C. The culture supernatants were assayed for diazepam concentration. The experiment was conducted in triplicate, and the mean value was calculated.

Statistical Analysis

Significance was evaluated by single-factor and more dependent variable analysis of variance by using Excel 2003 (Microsoft Corporation, Redmond, Wash, United States) and SPSS version 11.5 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, United States). A P value < .05 was considered statistically significant, and the data are expressed as mean values ± SDs.

RESULTS

Characteristics of Microcapsules

The microcapsules were round, with a diameter ranging from 350 to 450 μm and with many small pores over the surface. The microencapsulated HepLL and HepG2 cells exhibited the characteristics of small mature hepatocytes, as shown by transmission electron microscopy. They contain...
were no signifi-
cant differences between the numbers of HepLL and HepG2 cells according to different speeds and different densities ($P = .09$) (Table 2, Fig 4).

**Albumin Synthetic Function**

Albumin production in the HepG2 group rose rapidly from day 2, reaching the peak on day 10 ($0.36$–$1.62$ pg/cell per hour) and then declined rapidly, with only $0.02$ to $0.42$ pg/cell per hour on day 16. In the HepLL group, albumin production rose rapidly in the first 2 to 6 days, and then increased slowly; it reached the peak on day 10 ($0.35$–$1.49$ pg/cell per hour), followed by a slow decline after day 10 ($0.31$–$1.43$ pg/cell per hour on day 12) and a rapid decline between days 12 and 16 ($0.03$–$0.46$ pg/cell per hour on day 16). Overall, albumin production remained relatively stable between days 6 and 12. The results indicate that the cells significantly increased the amount of albumin synthesis with the speed increase (range, 0–4 rpm) ($P = .00$), with no effect of cell density culture on this production ($P = .83$). Encapsulated HepLL cells synthesized more albumin than embedded HepG2 cells at any time point ($P = .001$). Blank control groups were unable to detect albumin (Table 3, Fig 5).

**Ammonium Clearance**

Ammonium clearance was maintained during a 16-day period, with maximum rates on day 10 (Table 4, Fig 6). Ammonium removal of the microencapsulated HepG2 cells

![Encapsulated Cells Proliferation Curve](image)

**Fig. 4.** Microencapsulated cells proliferation. Solid line: HepLL group, dashed line: HepG2 group; Black: 0 RPM group, Green: 2 RPM group, Blue: 3 RPM group, Red: 4 RPM group; embedded cell density of 1.0 x 10^6 group, Δ: embedded cell density of 2.0 x 10^6 group. Encapsulated cell proliferation curve, Values are expressed as mean ± SD. The cell number in higher speed group was significantly greater than in the lower speed group during a 16-day period in 0 rpm - 4 rpm ($P = .00$). No differential effects of cell density on cell number were observed in each group ($P = .79$). No significant difference in the number of HepLL and HepG2 cells when cultured at different speeds and densities ($P = .09$).
quickly increased from day 2 to day 10 (0.1–1.21 pmol/100 cell/2 hours on day 2 vs 0.65–2.28 pmol/100 cell/2 hours on day 10). A rapid decrease was then observed; clearance at day 16 was only 0.08 to 2.24 pmol/100 cell/2 hours. Ammonium removal of the microencapsulated HepLL cells quickly increased from day 2 to day 8 (0.18–1.77 pmol/100 cell/2 hours on day 2 vs 0.93–6.59 pmol/cell/h on day 8), with the peak on day 10 (0.98–6.53 pmol/100 cell/2 hours). There was a relative plateau between days 8 and 12 and then a rapid decline. When comparing similar cells cultured at similar density, the activity per cell was markedly increased in the faster speed groups for both HepLL and HepG2 cells (\( P = .00 \)). No differential effects of cell density on ammonium removal were observed in encapsulated HepLL and HepG2 cell cultures (\( P = .60 \)). The activity of HepLL cells to remove ammonium was significantly higher than that of HepG2 cells at various time points (\( P = .00 \)). Ammonium chloride concentrations of the blank control group were maintained at 1 mmol/L at any time, regardless of any density or any speed.

**Diazepam Clearance**

Diazepam clearance of the microencapsulated HepG2 groups was 0.12 to 1.12 pg/cell/hour on day 2; it quickly rose, reaching the peak 1.75 to 7.49 pg/cell/hour on day 10 and decreased rapidly to 0.09 to 1.29 pg/cell/hour on day 16 (Table 5, Fig 7). Diazepam removal in the HepLL group rose rapidly between days 2 and 6 (0.41–3.68 pg/cell/hour on day 2 vs 1.56–8.78 pg/cell/hour on day 6), then slowly rose and reached the peak on day 10 (2.32–9.59 pg/cell/hour on day 10); it declining slowly on days 10 to 12 (2.01–9.14 pg/cell/hour on day 12), followed by a rapid decline. Overall, diazepam removal remained relatively stable between days 6 and 12, exhibiting kinetics similar to that of albumin secretion.

Diazepam clearance significantly increased with the speed increase of 0 to 4 rpm (\( P = .00 \)) but had no relationship with density (\( P = .57 \)). The ability of encapsulated cells to clear diazepam in all cases was significantly higher with HepLL cells than with HepG2 cells (\( P = .00 \)). Diazepam concentrations of the blank control group were maintained at 5 \( \mu \)g/L at any time, regardless of density and speed.

**DISCUSSION**

For each group, cells were quiescent on day 1, and this may be due to the long coating procedure required by the AC encapsulation (chitosan needs at least 30 minutes of exposure to complete cross-linking with the alginate...
molecules) [24]. The reduction in cell viability may be due to low pH and temperature and the limited supply of nutrients and carbon dioxide during this encapsulation phase.

Over the following 9 days, a logarithmic growth phase was observed in cell amplification. Cells recovered from the damage of the microcapsulation, adapting to the environment within the microcapsule and the adequate supply of nutrients. The concentration of metabolic byproducts remained limited, and energy exchange between cells and their environment was efficient. All these factors contributed to rapid cell proliferation between days 2 and 10. After day 10, cell numbers in all groups decreased. Several mechanisms may be involved: (1) microcapsulated cell expansion (with the increased volume of aggregates, passive diffusion barriers would limit the intracellular transport, and decreasing surface to volume ratio would affect the intake of essential nutrients for cell growth); (2) massive accumulation of metabolic byproducts (hindering nutrition exchanges between the cell and its environment); (3) microcapsule aging (limiting the penetration of nutrients); and (4) relative lack of nutrients in relation to the large quantity of cells.

In the range of 0 to 4 rpm, cell number and micro-encapsulated cell activity (eg. albumin synthesis, ammonia removal, diazepam clearance) were significantly greater in the higher speed group at each time point. There are several possible reasons for this finding. One factor may be the viscosity effect of lower speeds toward the microcapsules, which would be more prone to remain in the spinner wall and therefore not reach the culture medium. Second, with an appropriate increase, accelerated convection [25], increase of dissolved oxygen in the medium, better gas exchanges, with avoidance of turbulence and gas chamber caused by bubbles [26], nutrients and metabolites exchange inside and outside of microcapsules are improved. Third, with an appropriate speed, not all microcapsules are immersed in the culture medium, allowing easier access to oxygen for those on the surface. Thus, with the increase in speed, the microcapsules move with the liquid flowing. Roller bottles rotate around a horizontal axis; the microcapsules rotate similarly, and gravitational vectors may directly affect the intracapsular cell gene expression or indirectly contribute to the cell autocrine and paracrine processes, with an overall conductive effect to signal transduction. Lastly, cell access to some unrestricted 3-dimensional space favors cell–cell and cell–matrix interactions but also cell differentiation without a necrotizing center [27].

Currently, most spinner culture systems have been used for the preparation of biological products and not for...
Microencapsulated HepLL cell activity, as expressed by albumin synthesis, ammonia removal, and diazepam clearance, was higher in the rotation experiments compared with the stationary groups, highlighting the advantages of spinner culture. However, all these activities were minimal on day 18, indicating that microencapsulated HepLL cells had no long-standing activity. On day 14, we observed some broken microcapsules and escaping cells, indicating that there is still room for improvement of the microcapsule mechanical strength. It is clear that several conditions should be further optimized, such as microencapsulated material selection, wrapping procedure, and culture medium composition.

Cell proliferation rate and cell activity are known to be closely related with cell density [25–32] because each cultured cell occupies more extracellular medium at lower density than at higher density. Therefore, the number of ligands and the receptor-binding rate are much higher in low-density cell cultures, favoring cell signaling. However, receptors would become saturated when cellular density drops over a certain extent, with an excess of ligands, which would no longer be propitious to cell signaling [28]. On the contrary, it might have an adverse impact on the secretion of cytokines, which in turn would affect the growth and activity of cells.

Our results, however, show that encapsulated cell expansion rate and cell functions were not related to embedded cell density. This might be due to the small difference in our selected cell densities (compared with changes >10 times in the other studies) and/or different cell types [28–35].

Cheng et al [14] reported that embedded HepLL cells could synthesize albumin 6.77 ± 0.45 µg/day/10^7 cells on day 2, rising to the peak of 90.33 ± 2.85 µg/day/10^7 cells on day 12. In the present study, HepLL hepatocytes were able to synthesize more albumin, and encapsulated HepLL cells had the strongest activity on day 10, suggesting that cell viability may change along with the culture conditions and

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**Table 5. Diazepam Clearance of Encapsulated Cells**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 10</th>
<th>Day 12</th>
<th>Day 14</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1</td>
<td>0.41 ± 0.05</td>
<td>0.53 ± 0.04</td>
<td>1.56 ± 0.17</td>
<td>2.27 ± 0.16</td>
<td>2.32 ± 0.25</td>
<td>2.01 ± 0.19</td>
<td>1.07 ± 0.14</td>
<td>0.73 ± 0.05</td>
</tr>
<tr>
<td>A_2</td>
<td>0.12 ± 0.01</td>
<td>0.28 ± 0.03</td>
<td>0.77 ± 0.12</td>
<td>1.19 ± 0.08</td>
<td>1.75 ± 0.13</td>
<td>0.34 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>A_3</td>
<td>1.34 ± 0.15</td>
<td>2.57 ± 0.19</td>
<td>5.32 ± 0.37</td>
<td>5.69 ± 0.34</td>
<td>6.18 ± 0.38</td>
<td>5.83 ± 0.36</td>
<td>3.9 ± 0.22</td>
<td>2.38 ± 0.19</td>
</tr>
<tr>
<td>A_4</td>
<td>0.42 ± 0.07</td>
<td>1.72 ± 0.15</td>
<td>2.83 ± 0.23</td>
<td>3.72 ± 0.28</td>
<td>4.37 ± 0.32</td>
<td>2.19 ± 0.23</td>
<td>0.79 ± 0.09</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>A_5</td>
<td>2.28 ± 0.19</td>
<td>4.31 ± 0.31</td>
<td>7.23 ± 0.46</td>
<td>7.31 ± 0.39</td>
<td>7.71 ± 0.41</td>
<td>7.28 ± 0.45</td>
<td>5.13 ± 0.33</td>
<td>2.67 ± 0.19</td>
</tr>
<tr>
<td>F_1</td>
<td>0.68 ± 0.04</td>
<td>2.12 ± 0.18</td>
<td>4.15 ± 0.33</td>
<td>4.91 ± 0.27</td>
<td>6.23 ± 0.49</td>
<td>3.15 ± 0.27</td>
<td>1.33 ± 0.23</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>E_1</td>
<td>3.41 ± 0.27</td>
<td>5.23 ± 0.29</td>
<td>8.37 ± 0.56</td>
<td>8.49 ± 0.62</td>
<td>9.12 ± 0.55</td>
<td>8.63 ± 0.59</td>
<td>5.96 ± 0.38</td>
<td>3.07 ± 0.26</td>
</tr>
<tr>
<td>F_2</td>
<td>0.87 ± 0.12</td>
<td>3.34 ± 0.26</td>
<td>5.28 ± 0.39</td>
<td>6.33 ± 0.41</td>
<td>7.07 ± 0.47</td>
<td>4.98 ± 0.36</td>
<td>2.16 ± 0.14</td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>G_1</td>
<td>0.57 ± 0.06</td>
<td>0.83 ± 0.12</td>
<td>1.97 ± 0.16</td>
<td>2.25 ± 0.18</td>
<td>2.54 ± 0.22</td>
<td>2.13 ± 0.19</td>
<td>1.31 ± 0.15</td>
<td>0.82 ± 0.03</td>
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<tr>
<td>D_1</td>
<td>0.3 ± 0.04</td>
<td>0.53 ± 0.06</td>
<td>0.89 ± 0.08</td>
<td>1.32 ± 0.11</td>
<td>1.98 ± 0.16</td>
<td>0.92 ± 0.12</td>
<td>0.19 ± 0.04</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>G_2</td>
<td>1.68 ± 0.21</td>
<td>2.19 ± 0.16</td>
<td>4.93 ± 0.36</td>
<td>5.34 ± 0.46</td>
<td>5.51 ± 0.38</td>
<td>5.27 ± 0.36</td>
<td>3.5 ± 0.21</td>
<td>2.09 ± 0.31</td>
</tr>
<tr>
<td>H_1</td>
<td>0.75 ± 0.09</td>
<td>1.93 ± 0.21</td>
<td>3.09 ± 0.21</td>
<td>4.12 ± 0.33</td>
<td>4.78 ± 0.32</td>
<td>1.72 ± 0.14</td>
<td>1.02 ± 0.07</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>G_3</td>
<td>2.27 ± 0.11</td>
<td>4.67 ± 0.32</td>
<td>6.85 ± 0.32</td>
<td>7.52 ± 0.36</td>
<td>8.11 ± 0.54</td>
<td>7.76 ± 0.51</td>
<td>4.88 ± 0.26</td>
<td>2.36 ± 0.13</td>
</tr>
<tr>
<td>H_2</td>
<td>0.57 ± 0.06</td>
<td>2.56 ± 0.23</td>
<td>3.71 ± 0.18</td>
<td>4.85 ± 0.26</td>
<td>5.73 ± 0.37</td>
<td>2.48 ± 0.17</td>
<td>1.63 ± 0.11</td>
<td>0.85 ± 0.09</td>
</tr>
<tr>
<td>G_4</td>
<td>3.68 ± 0.21</td>
<td>5.78 ± 0.32</td>
<td>8.78 ± 0.46</td>
<td>9.21 ± 0.47</td>
<td>9.59 ± 0.51</td>
<td>9.14 ± 0.49</td>
<td>6.34 ± 0.36</td>
<td>3.62 ± 0.25</td>
</tr>
<tr>
<td>H_3</td>
<td>1.12 ± 0.09</td>
<td>3.63 ± 0.25</td>
<td>4.91 ± 0.23</td>
<td>6.59 ± 0.34</td>
<td>7.49 ± 0.46</td>
<td>4.47 ± 0.29</td>
<td>2.85 ± 0.24</td>
<td>1.29 ± 0.08</td>
</tr>
</tbody>
</table>

*Data are expressed as mean values ± SDs (µg/cell/h).*

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**Fig. 7.** Diazepam clearance. Solid line: HepLL group, dashed line: HepG2 group; Black: 0 RPM group, Green: 2 RPM group, Blue: 3 RPM group, Red: 4 RPM group; □: embedded cell density of 1.0 × 10^6 group, △: embedded cell density of 2.0 × 10^6 group. Comparison of diazepam clearance of embedded hepatocytes cultured with various spinner rates. Concentrations are described as picograms per cell per hour (mean ± SD). The mean diazepam clearance in higher speed group was significantly greater than in the lower speed group during a 16-day period in 0–4 RPM (P = .00), but had no relationship with density (P = .57). The ability of encapsulated HepLL cells to clear diazepam in all cases was significantly higher than that of HepG2 cells (P = .00).
embedding parameters. HepG2 cells have been extensively studied as the most commonly used bioartificial liver cells. Our study found that microcapsule HepLL cells not only have stronger activity than embedded HepG2 cells, but also that their activity duration was significantly longer than microcapsule HepG2 cells. Therefore, using HepLL instead of HepG2 may represent a new option for bioartificial liver.

In conclusion, spinning culture significantly promoted the microencapsulated HepLL and HepG2 cell activity. In the range of 0 to 4 rpm, albumin synthesis, ammonia removal, and diazepam clearance gradually increased with increases in speed, with no special interference of embedded cell density. Wrapped HepLL cells had optimal function on day 10 in rolling culture groups. Our data could pave the way for setting up appropriate liver cell support in patients with severe liver failure.

REFERENCES


[2] Chamuleau RA, Deurholt T, Hockstra R. Which are the right cells to be used in a bioartificial liver? Metab Brain Dis 2005;20:327–35.


