INTRODUCTION

Technologies necessary for the development of bioartificial liver tissue must necessarily involve approaches to engineer both cell proliferation, as an endogenous means to provide replenishable cell sources,1–3 as well as cell differentiation, to replace the function of the diseased organ. Many studies have focused on modulation of these processes in hepatocytes, the parenchymal cells of the liver, via tunable parameters including...
cell density, growth factor stimulation, chemical and mechanical properties of the extracellular matrix and cocultivation with other cell types. Studies adjusting these parameters to achieve proliferation or differentiation have met with some success, but observed that an enhancement in one process is associated with a decline in the other, and thus that there exists a delicate balance between proliferation and differentiation. These studies, however, examine secondary effects of phenotypological outcomes resulting from a combination of disparate parameters. To properly engineer the balance between these converse cellular states, a more focused molecular strategy to alter cellular processes may be warranted. Thus, an understanding of effects on the differentiation-proliferation balance (DPB) of molecules mediating key cellular parameters such as cell-cell and cell-matrix contacts is critical to the design of cell-based systems.

Previous studies have demonstrated a role for cell-cell contact in the modulation of proliferation and differentiation. For example, increasing amounts of cell-cell contact exhibit marked increases in liver-specific function, whereas growth is often inhibited by cell-cell contact in dense cultures. Our previous studies have demonstrated a liver-specific function-inductive role for E-cadherin, a homotypic cell-cell adhesion receptor mediating cell-cell contacts, in a model system based on heterocellular cadherin presentation. In addition, it has been demonstrated that purified recombinant cadherins can induce muscle-specific transcripts and decrease the number of cells in S phase when applied to myoblasts by a solid support. Whereas the role of cellular cadherins in the control of differentiated hepatocyte function has been established, the role in the control of the DPB of acellular cadherins with relevance to biomaterial design for liver cells requires further investigation.

In this study, we have employed a microsphere-based oriented cadherin presentation model system (Figs. 1 and 2) to examine the possible modulation of hepatocellular DPB by display of acellular cadherins to hepatocytes immobilized on a collagen gel (Fig. 2). Further, we examined hepatocyte cocultures engaged via endogenous cell-based cadherins (lateral domain), using the exogenous display of our basally oriented acellular cadherin model. We report that acellular cadherin presentation results in an induction of hepatocellular DNA synthesis and cell division. Furthermore, we observed a simultaneous decrease in albumin messages, a transcriptionally controlled marker of hepatocellular differentiation, indicating that the DPB is shifted toward a proliferative state by acellular cadherins. When acellular cadherins were applied to hepatocytes in the cellular cadherin model system, the enhancement of hepatocyte function is reversed such that there is no longer a coculture-related functional induction. Thus, cadherin presentation, either in cellular or acellular form, represents a new molecular strategy to modulate the DPB within hepatocyte cultures, and may potentially afford a key route to engineer sustainable hepatic tissues in the future.

**MATERIALS AND METHODS**

**Cell isolation and culture**

Hepatocytes were isolated from anesthetized Fischer male rats in accordance with a published protocol, the Rutgers Institutional Review Board Guidelines for the Use and Care of Animals, as well as Protocol Review Number 97-001. Isolated hepatocytes were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Mediatech, Herndon, VA) containing penicillin, streptomycin, glutamine, heat-inactivated fetal bovine serum (Cambrex Bio Science Walkersville, Walkersville, MD), insulin, glucagon, hydrocortisone, and epidermal growth factor (Sigma, St. Louis, MO) (C1H medium). Hepatocytes were seeded onto gelled rat tail collagen type I at a density of $1.0 \times 10^5$ cells/cm$^2$ for microscopy and functional gene expression studies and at a density of $2.0 \times 10^5$ cells/cm$^2$ for studies on cell proliferative potential. Immediately after cell seeding, $4.75 \times 10^6$ cadherin-functionalized or control microspheres/cm$^2$ (prepared as described below) were added to the cultures. Medium was exchanged daily. One day after culture preparation, culture images were captured using transmitted light on a laser-scanning inverted confocal microscope (Carl Zeiss, Thornwood, NY).

For cocultures, hepatocytes were seeded onto collagen gels at a density of $1.2 \times 10^4$ cells/cm$^2$. The next day, growth of cadherin-presenting or control (cadherin-lacking) fibroblastic chaperone cells was arrested with mitomycin C (10 μg/mL; Sigma), dissociated with 0.1% trypsin (Cambrex Bio Science Walkersville) in the presence of 1.2 mM CaCl$_2$, and added to hepatocyte cultures at a density of $1.1 \times 10^5$ cells/cm$^2$. After the passing of an additional day $4.8 \times 10^6$ microspheres/cm$^2$ (functionalized as described below) were applied to the cocultures. Medium was removed daily, saved for later analysis, and replaced with fresh medium.

**Microsphere functionalization**

A microsphere-based cadherin presentation system was designed to present active cadherin fragments to hepatocytes (Fig. 1). Acellular cadherin dimerization, a necessary condition for cadherins on opposing surfaces to interact, was driven by two disulfide bonds within the Fc region of human IgG that was fused to the five extracellular subdomains of mouse E-cadherin (Fig. 1). Orientation of acellular cadherins on the microsphere surface for interaction with hepatocellular cadherins was...
accomplished by the specific binding of the Fc domain to protein A from Staphylococcus aureus, which was covalently attached the microspheres (Fig. 1). We designed acellular cadherin presentation via microbeads to hepatocytes cultured on a collagen gel (Fig. 2). The cadherin overlay was employed instead of a cadherin-based underlay to avoid the cell morphogenesis that occurs when hepatocytes are seeded on cadherin-derivatized microbeads (data not shown).

Microspheres with covalently bound protein A, 5.5 μm in diameter (Bangs Laboratories, Fishers, IN), were washed twice with 50 mM sodium borate (FisherBiotech; Fisher Scientific Chemical Division, Fair Lawn, NJ), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.2, containing 0.5% Pluronic-F68 (ABB) (Sigma). After a 10-min sonication, beads were treated with double the binding capacity (reported by Bangs Laboratories) of mouse E-cadherin/human IgG Fc chimeras (R&D Systems, Minneapolis, MN) diluted in ABB. As a control, beads were exposed to an equal molar amount of purified human IgG Fc fragments (ICN Biomedicals, Aurora, OH). After 1 h of cadherin or control coating at room

FIG. 1. Oriented presentation of acellular cadherin dimers. Chimeras of the five cadherin extracellular subdomains (parallel lines) fused to the Fc region of human IgG (shaded) dimerize through the two disulfide bonds in the Fc region, rendering them adhesion competent. Specific adhesion of the Fc region to protein A (criss-crossed) covalently attached to the microbead surface results in proper chimera orientation.

FIG. 2. Application of cadherin-presenting microbeads to hepatocytes. Hepatocytes cultured on a collagen gel are treated with microbeads at their basal (upper) domain (A). Transmitted image of hepatocytes cultured for 1 day with microbeads (B). Horizontal arrow indicates the cell–cell borders of a hepatocyte overlaid with beads; vertical arrow indicates the cadherin-derivatized beads. Direct viewing of hepatocytes is obscured by the bead overlay. Scale bar, 100 μm.
temperature, beads were washed twice with ABB and then exposed to Fc for an additional 1 h to block any unbound protein A sites. Beads were stored at 4°C in 100 mM sodium borate, 10 mM EDTA, pH 8.5, containing 0.5% Pluronic-F68 and 0.1% sodium azide. Beads were washed five times with C+H medium, sterilized with ultraviolet light, and sonicated before use.

To confirm the presence of cadherins on the bead surface, beads were stained with rabbit antiserum to E-cadherin (gift of R. Kemler) and a mouse anti-rabbit IgG–Texas Red conjugated (Jackson ImmunoResearch, West Grove, PA). After staining, beads were analyzed in a flow cytometer (BD Biosciences Transduction Laboratories, Lexington, KY).

**Measurement of hepatocellular proliferative potential**

To evaluate the proliferative potential of hepatocytes, we chose to monitor the two key processes, DNA synthesis and cell division (Fig. 3). As these processes may not invariably occur in adult rat hepatocytes in vitro, causing the accumulation of highly polyploid cells (Fig. 3),

![Diagram of DNA synthesis and cell division](image)

We chose to measure both simultaneously. To that end, we quantified newly synthesized DNA by bromodeoxyuridine (BrdU) labeling and scored cells into cell cycle stages based on the amount of DNA present in each cell, as measured by propidium iodide staining. Briefly, cell cultures were prepared as described above and after 3 days of culture, cells synthesizing DNA were labeled by incubation for 5 h with 5-bromo-2'-deoxyuridine (ICN Biomedicals) in C+H medium. After labeling, cells were detached by incubation in trypsin–EDTA (Sigma) and fixed in ethanol. Cells were incubated for 30 min in RNase (10 μg/mL; Sigma) in phosphate-buffered saline (PBS) at room temperature. After digestion of RNA, DNA was denatured by incubation at room temperature for 30 min in 2 N HCl, 0.5% Triton X-100 (Fisher-Biotech; Fisher Scientific Chemical Division) and then washed with 0.1 M sodium borate, pH 8.5. Staining was accomplished by the addition of a 1:100 dilution of an anti-BrdU–fluorescein isothiocyanate (FITC) conjugate (BD Biosciences Pharmingen, San Diego, CA) in 0.5% Tween 20 (FisherBiotech; Fisher Scientific Chemical Division) in PBS (PBS-T). After one wash in PBS-T, total cellular DNA was stained with propidium iodide (15 μg/mL; Sigma) in PBS and analyzed at the Flow Cytometry Core Facility at Rutgers University (Piscataway, NJ). Cells were counted as BrdU labeled if their FITC fluorescence were more than 1 unit greater than the mean fluorescence of the peak on the histogram representing unlabeled cells. Histograms of propidium iodide-labeling exhibited two peaks, the higher of which had double the fluorescence of the lower one (data not shown). A small peak was observed at a fluorescence level four times that of the lowest fluorescence peak (data not shown). The cells exhibiting the smallest fluorescence were considered to be cells with a 2N amount of DNA (cells in G0 or G1 of the cell cycle) (Fig. 3), the cells exhibiting double the smallest amount were considered to have a 4N amount of DNA (cells in the G2 phase of the cell cycle, which have synthesized DNA, but have not yet undergone cell division) (Fig. 3). As the number of cells with an 8N amount of DNA (cells that have completed two rounds of DNA synthesis without cell division) and cells containing intermediate amounts of DNA (cells that have

**FIG. 3.** Theoretical scheme for the varied DNA content of hepatocytes underlying the hepatocyte proliferative potential. Under ideal conditions, DNA synthesis results in a 2-fold increase in DNA content per cell, whereas mitosis followed by cytokinesis results in a reduction in cellular DNA by one-half. Cells that do not undergo cell division after DNA synthesis retain double the amount of DNA as cells that undergo cell division. We measure DNA synthesis by BrdU incorporation and cell division (cytokinesis) via differences in propidium iodide staining (data reported in Fig. 5). DNA content is indicated below each cell.
not completed DNA synthesis) were small (data not shown), cells were scored as having either a 2N amount of DNA (2N cells) or a greater than 2N amount of DNA (>2N cells). As cells were double stained, we also were able to determine which subsets of labeled cells were 2N cells or >2N cells by simultaneously applying both criteria.

**Measurement of secreted products**

Medium samples collected from daily medium exchanges were assayed for secreted albumin by an enzyme-linked immunosorbent assay (ELISA). Assay plates were formed by overnight incubation at 4°C of Maxisorp plates (Nalge Nunc International, Rochester, NY) with purified rat albumin (ICN Biomedicals) in 15 mM Na2CO3 and 35 mM NaHCO3 (Sigma), pH 9.6. Samples were incubated in assay plates overnight at 4°C in the presence of anti-rat albumin–horseradish peroxidase conjugate (ICN Biomedicals). The following day, after reacting plates in hydrogen peroxide and o-phenylenediamine dihydrochloride (Sigma) for 5 min and stopping the reaction with 8 N sulfuric acid (Fisher Biotech; Fisher Scientific Chemical Division), absorbance was measured at 490 nm with a microplate reader (Bio-Rad, Hercules, CA). Concentrations were calculated from absorbances of known concentrations of purified rat albumin.

Urea levels in medium samples were measured with a commercially available colorimetric assay kit (Sigma) modified to fit a 96-well plate and a microplate reader detecting absorbance at 490 nm.

**Quantitation of hepatocellular function via gene expression**

A kinetic reverse transcriptase-polymerase chain reaction (RT-PCR) assay was employed to evaluate the mRNA levels of albumin and a housekeeping gene, 18S ribosomal RNA. Briefly, cell cultures were established as described above and, after the passing of 0.5, 3, or 6 days, RNA was isolated from cell cultures and DNA contamination was digested with a silica column and a DNase-based High Pure RNA isolation kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions and frozen until later use. Samples (5 μL) of RNA were reverse transcribed with the lower primer (see below) and an Access RT-PCR kit (Promega, Madison, WI). The resulting cDNA was amplified with a Quantitect SYBR Green PCR kit (Qiagen, Valencia, CA) on a Light Cycler (Roche Diagnostics; courtesy of C.M. Roth, Molecular Bioengineering Laboratory, Rutgers University) with an annealing temperature of 56°C. Primers were designed on the basis of the literature29: upper (5’-TGAGAACCAGGCCACTATCTC) and lower (5’-CT-CAGCAGAGGACACCGAGTAA) primers for albumin and upper (5’-CCCGAGCCACCTGGTAC) and lower (5’-CCAGTGCCGCTGTTTAT) primers for 18S ribosomal RNA. Albumin expression is normalized to the corresponding expression of 18S ribosomal RNA.

**Statistics**

The standard error was calculated on the basis of replicate samples and reported as error bars. Statistics were analyzed with a significance test comparing two measurements of known variance based on a normal distribution. Data was accepted as significant for $p < 0.05$.

**RESULTS**

**Microsphere-based oriented acellular cadherin model system**

Dimerized recombinant cadherins were presented via polymeric microspheres to primary rat hepatocytes immobilized on a collagen gel (Figs. 1 and 2). The presence of cadherins on the bead surface was confirmed by indirect immunofluorescent staining for E-cadherin along with flow cytometric analysis. Cadherin/Fc chimera-functionalized beads exhibited a higher fluorescence than control, Fc-functionalized beads (Fig. 4). In particular, 90.9% of cadherin/Fc-presenting beads had a fluorescence of 50 or more, whereas only 0.4% of Fc-presenting beads exhibited a fluorescence of 50 or more (Fig. 4).

**Effect of cadherin-presenting beads on hepatocellular proliferative potential**

To evaluate the role in hepatocellular proliferation of acellular cadherin presentation, we cultured hepatocytes in the presence of cadherin/Fc chimera-functionalized microbeads and analyzed hepatocyte DNA synthesis and cell cycle behavior via flow cytometry. Cells were scored on whether or not they incorporated BrdU and as either

![FIG. 4. Flow cytometric analysis of cadherin/Fc chimera-presenting (solid line) and Fc-presenting (dashed line) microbeads stained with antibodies to E-cadherin.](image-url)
having a 2N amount of DNA (2N cells) or a greater than 2N amount of DNA (>2N cells) (see Materials and Methods). The percentage of cadherin/Fc chimera-exposed hepatocytes positive for bromodeoxyuridine incorporation (18% of total cells) was greater than that of Fc-exposed hepatocytes (11% of total cells) (p < 0.05) (Fig. 5). Hepatocyte cultures treated with cadherin/Fc chimera-presenting beads had more 2N cells (27%) than did cultures treated with control, Fc-presenting beads (16%) (p < 0.05) (Fig. 6). Conversely, cadherin/Fc-exposed cultures had fewer >2N cells (73%) compared with Fc-exposed cultures (84%) (p < 0.05) (Fig. 6). By simultaneously

**FIG. 5.** Effect of cadherin presentation on hepatocyte DNA synthesis. The percentage of total hepatocytes incorporating bromodeoxyuridine when treated with cadherin/Fc chimera-presenting beads (shaded columns) or Fc-presenting beads (open columns). Also, the percentage of BrdU-labeled cells that possess either a 2N or >2N amount of DNA, based on propidium iodide labeling, is reported. *Significant difference from Fc-treated cells (p < 0.05); #significant difference in labeling from >2N cells.

**FIG. 6.** Effect of cadherin presentation on the hepatocyte cell cycle. Shown is the percentage of cells with a 2N (shaded columns) or greater than 2N (open columns) amount of DNA in response to treatment with cadherin/Fc chimera-functionalized beads or control, Fc-functionalized beads. *Significant difference (p < 0.05) between cells treated with cadherin/Fc chimera-functionalized beads and cells treated with Fc-functionalized beads.
measuring cellular DNA content and BrdU labeling, we determined which phases of the cell cycle contained newly synthesized DNA (see Materials and Methods). In hepatocyte cultures exposed to cadherin/Fc microbeads, BrdU labeling was distributed similarly between 2N cells (55% of BrdU-labeled cells had a 2N amount of DNA) and >2N cells (45% of BrdU-labeled cells had a >2N amount of DNA) (Fig. 5). In hepatocyte cultures treated with Fc microbeads, we observed that BrdU-labeled cells more often contained a >2N amount of DNA (77% of BrdU-labeled cells had a >2N amount of DNA) than a 2N amount of DNA (23% of BrdU-labeled cells had a 2N amount of DNA) (p < 0.05) (Fig. 5).

Taken together, our DNA synthesis and cell cycle data indicate that hepatocytes exposed to cadherins have a greater proliferative potential than control, Fc-exposed hepatocytes. In particular, greater BrdU labeling for cultures treated with cadherin/Fc chimera beads indicates greater DNA synthesis. Further, a greater amount of 2N cells indicates that these cells also experience a greater number of cell divisions, as cell division results in a reduction by half of DNA in each cell, and failure of cell division results in a 2-fold increase in cellular DNA (Fig. 3). In addition, because BrdU-labeled cells in cadherin-treated cultures more often had a 2N amount of DNA than did control cultures, these cells likely underwent cell division after DNA synthesis. Finally, our observation of equivalent DNA synthesis and cell cycle trends on day 6 (data not shown) suggests that the cadherin-mediated induction of hepatocyte proliferative potential is a long-term phenomenon.

Effect on liver-specific function of cadherin-presenting beads

We sought to quantify the functional response of hepatocytes to cadherin presentation. To that end, we cultured cells in the presence of microspheres and measured the levels of mRNA transcripts of albumin, a marker of liver-specific function, using a kinetic RT-PCR assay. Cadherin-functionalized beads caused a statistically significant time-averaged decrease of almost 40% in the level of albumin transcripts relative to nonfunctionalized beads over the 6-day culture period (Fig. 7). Fc-functionalized beads did not cause a statistically significant change in albumin message levels relative to nonfunctionalized beads (Fig. 7).

Because acellular cadherin-mediated repression of liver-specific function contrasts with the inductive effects of cellular cadherins reported previously, we next sought to determine the interplay of both presentation mechanisms simultaneously. To that end, we formed cocultures of hepatocytes with cadherin-expressing or deficient chaperones and added cadherin/Fc- or Fc-presenting microspheres. To evaluate the effect on function of the presence of the cadherin-functionalized microspheres, we measured

FIG. 7. Functional response of hepatocytes to microbead presentation. Albumin message levels, normalized to 18S RNA, of hepatocytes exposed to cadherin/Fc chimera-functionalized microbeads (■) and Fc-functionalized microbeads (▲) relative to nonfunctionalized beads. *Significant difference (p < 0.05) between cells treated with cadherin/Fc chimera-functionalized beads and cells treated with Fc-functionalized beads.
the percent change in bead-treated cocultures relative to cocultures lacking beads, of two markers of hepatocyte differentiation: long-term (average of days 5–8) secretion of albumin and urea. Acellular cadherin-presenting beads caused a 47% percent decrease in secreted albumin in cellular cadherin cocultures and a 42% decrease in control, cellular cadherin-deficient cocultures (Fig. 8). The presence of Fc-presenting beads did not cause a statistically significant change in albumin secretion of cocultures (Fig. 8). Acellular cadherin-presenting beads caused a greater decrease in secreted urea in cellular cadherin cocultures than Fc-presenting beads and a 29% decrease in secreted urea in control, cellular cadherin-deficient cocultures, whereas Fc beads caused no statistically significant change in urea secretion (Fig. 9).

**DISCUSSION**

The ability to engineer the hepatocellular differentiation–proliferation balance (DPB) is critical to the development of functional organ replacement devices as well as sources for their component cells. We have investigated the role of E-cadherin, a key cell–cell adhesion molecule, in the control of the DPB, with a view toward providing the basis to design coculture and biomaterial-based systems. We demonstrate that microsphere-presented acellular cadherins induce an increase in hepatocyte DNA synthesis and proliferative potential accompanied by a decrease in liver-specific function-governing transcripts. The other major finding of our study is that the functional role of cadherin is dependent on the mode of cadherin presentation, as we observed contrasting DBP results for acellular cadherins when compared with previous studies on cell-based cadherins. Notably, the DPB outcomes of simultaneous presentation of cellular and acellular cadherins were largely dominated by the exogenous cadherins.

We designed this study with a view toward extending insights from our highly functional cell-based cadherin cultures to biomaterials functionalized with acellular cadherins. We engineered, on the basis of previous studies, an efficient presentation of active cadherins by the use of protein A, an adapter molecule, which, via site-specific adhesion, correctly orients premixed, and therefore adhesion-competent, cadherin chimeras. Our results indicate that presentation of acellular cadherins can alter the DPB of hepatocytes, but interestingly, the switch in the DPB is inclined toward the proliferative regimen as opposed to the differentiation regimen. Specifically, our data show that DNA synthesis and proliferation potential were greater in cadherin-exposed hepatocytes, indicating a shift toward increased prolifer-

**FIG. 8.** Effect of acellular cadherin presentation on liver-specific function of hepatocytes cocultured with cadherin (+) or cadherin (−) chaperone cells. The change in hepatocyte albumin secretion resulting from micro bead exposure is reported relative to corresponding beadless cocultures. Single significant difference from cadherin (+) cocultures treated with Fc-functionalized beads; #significant difference from cadherin (−) cocultures treated with Fc-functionalized beads.
ation competence. This is consistent with previous investigations indicating a role for cadherins in the regulation of tumor growth and control of proliferation in cell lines. The increase in mitosis and cytokinesis may prove to be especially novel for tissue engineering, as hepatocytes often fail to undergo these processes in vitro. We also observed that the levels of albumin mRNA, an indicator of hepatocyte function, were reduced by cadherin presentation. A role for cadherins in the regulation of hepatocyte differentiated function is consistent with our previous studies, as well as studies demonstrating a role for cadherins in the control of differentiated function in other cell types. Taken together, our results indicate that cadherins induce an increase in proliferative potential, which arises at the expense of differentiation. This inverse correlation is in agreement with previous studies that altered cell–cell interactions, cell–matrix interactions, and cell shape to concertedly control the DPB. Specifically, treatments resulting in an increase in DNA synthesis resulted in a decrease in albumin secretion, and increases in albumin secretion were accompanied by a decrease in DNA synthesis. This inverse correlation of proliferation and function in hepatocytes is also qualitatively supported by reports that the induction of hepatocyte proliferation during regeneration or cell division results in low levels of liver-specific function, whereas a histone acetylation-induced decrease in hepatoma proliferation is accompanied by an increase in albumin and liver-specific transcription factors and differentiation involves exit from the cell cycle.

The influence exerted by acellular cadherins on the direction of shifts in DPB (i.e., decreasing the level of differentiation and increasing proliferative potential) is intriguing in view of the previous studies on cadherins. In particular, our own studies on the function of rat hepatocytes in response to chaperone cell-presented cellular cadherins indicated an increase in liver-specific function, whereas forced expression of cellular cadherins in other cell types induced markers of differentiation and a decrease in proliferation. Further, acellular N-cadherin-presenting beads induce differentiation and reduce DNA replication in myoblasts. It should be noted, however, that the mode of cadherin introduction and the interplay of exogenous cadherins with native cellular cadherins within the current study differ from most of the previous studies, thus allowing us to report on new insights into the modulatory aspect of cadherin regulation of the DPB. There may be several factors underlying the difference in hepatocyte response between cellular and acellular cadherins.

One explanation for the presentation-based difference
in hepatocellular response to cadherins is related to the polarization of hepatocytes. Specifically, hepatocytes are highly polarized cells with cadherin concentration at lateral surfaces of cell–cell contact.\textsuperscript{42} In our acellular cadherin presentation model system, cadherins are presented to the upper, basal surface of hepatocytes, possibly causing a differing response than to cadherins presented to the appropriate domains as in our coculture cellular cadherin presentation system\textsuperscript{22} or by other hepatocytes. Perhaps the hepatocellular response to cadherins presented at nonnative domains induces the disassembly of existing cadherin junctions as observed for dominant negative cadherins.\textsuperscript{43–45} resulting in a reduction in cadherin signaling and thus the converse effect of cadherins presented at lateral domains. This polarization dependence of acellular cadherin cellular guidance might not be as important in less differentiated cells, such as myoblasts, which had a junction assembly-related response.\textsuperscript{23}

An obvious difference between our acellular and cellular cadherin presentation systems is the presence of cell-based cosignaling possible in the chaperone system, raising the possibility of some other factor introduced by the chaperone cells that determines the direction of effect on the DPB by cadherins. Candidate factors include secreted molecules, extracellular matrix modifications, and chaperone cell membrane-associated molecules. Secreted factors are unlikely effectors of hepatocyte function, as coculture effects on hepatocytes could not be duplicated by conditioned medium and persist in cocultures with convection,\textsuperscript{14} and coculture effects on hepatocytes due to extracellular matrix-secreted molecules are inconclusive.\textsuperscript{15} In addition, a costimulatory role for secreted factors and extracellular matrix modifications can be further ruled out because acellular cadherins reduced hepatocellular function in cocultures containing these factors (Figs. 8 and 9), whereas cellular cadherins induced hepatocyte function under the same conditions.\textsuperscript{22} The most likely candidates for factors that might be involved in the hepatocyte response to cellular cadherins are cell membrane-associated molecules. In particular, the epidermal growth factor (EGF) receptor,\textsuperscript{46} calcium stretch channels,\textsuperscript{47} and a protein that was demonstrated to modulate hepatocyte function in cocultures, liver-regulating protein (LRP),\textsuperscript{48} are all potential coreceptors with cadherins. The presence of coreceptors on the chaperones but not on microbeads is consistent with such a mechanism. Efforts are currently underway in other laboratories to elucidate the molecular nature of cofactors.\textsuperscript{49}

One possible explanation for the contrast in cellular response between differing cadherin presentation mechanisms might be that our acellular cadherin presentation system strongly binds cadherins to the bead surface by protein A, restricting cadherin mobility. As cadherin reorganization into clusters is a key event in junction formation,\textsuperscript{23,30,51} limited mobility may hinder proper recruitment and assembly of the molecular factors at junctions, ultimately resulting in a change in signaling. The cadherin zipper is predicted by crystallographic data to be a lattice of trans-dimers separated by 7.5 nm.\textsuperscript{52} In our system, from first principles we estimate the average molecule spacing on the bead surface to be a little larger, about 10–20 nm, which may not allow proper lattice formation of both cadherins and their junctional proteins. Our data on hepatocytes exposed to cadherins presented by both mechanisms simultaneously support the possibility of acellular cadherins interrupting cadherin assembly and signaling, as the disassembly induced by acellular cadherins dominated. It is possible that the presentation of acellular N-cadherin is resistant to geometry-induced disassembly signals, as they might possess a subtly different lattice geometry or be less affected by geometric restrictions.

We investigated the effects on hepatocytes of cadherin homotypic cell–cell adhesion molecules with a view to develop ways to control the DPB in biomedical devices. Our results are particularly interesting because we discovered that this ligand can produce opposing effects, depending on the presentation mechanism, thus making it versatile. This versatility introduces the possibility of changing the differentiation and proliferative state of a culture simply by applying and removing acellular cadherins, either by adding and removal of microspheres, or by culturing directly on cadherin-functionalized biomaterials. Alternatively, a culture might be arranged in a biological microelectromechanical system into different regions of cells, some of which are in a proliferative state and serve as cell sources, while others are in a differentiated state, serving as the functional component of a device. Finally, with further understanding of the mechanism of cellular and acellular cadherin signaling interactions, it might be possible to engineer a balance of the two, maintaining cells within hepatocyte cultures in a state of simultaneous proliferation and function as has been demonstrated with a protein extract of royal jelly.\textsuperscript{53}

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