Studying host-pathogen interactions in 3-D: Organotypic models for infectious disease and drug development

Cheryl A. Nickerson, Ph.D.[1*], Emily G. Richter[1], C. Mark Ott, Ph.D. [2]

[1] Center for Infectious Diseases and Vaccinology

The Biodesign Institute

Arizona State University

Tempe, AZ 85287-5401

[2]Habitability and Environmental Factors Office

NASA-Johnson Space Center

Houston, TX 77058

*Corresponding Author

Center for Infectious Diseases and Vaccinology

The Biodesign Institute

Arizona State University

PO Box 875401

Tempe, AZ 85287-5401

Keywords: Three-dimensional (3-D) cell culture, physiologically relevant models, host-pathogen interaction, infectious disease, drug discovery

Summary

Representative, reproducible and high-throughput models of human cells and tissues are critical for a meaningful evaluation of host-pathogen interactions and are an essential component of the research developmental pipeline. The most informative infection models - animals, organ explants and human trials - are not suited for extensive evaluation of pathogenesis mechanisms and screening of candidate drugs. At the other extreme, more cost effective and accessible infection models such as conventional cell culture and static co-culture may not capture physiological and three-dimensional aspects of tissue biology that are important in assessing pathogenesis, and effectiveness and cytotoxicity of therapeutics. Our lab has used innovative bioengineering technology to establish biologically meaningful 3-D models of human tissues that recapitulate many aspects of the differentiated structure and function of the parental tissue *in vivo*, and we have applied these models to study infectious disease. We have established a variety of different 3-D models that are currently being used in infection studies - including small intestine, colon, lung, placenta, bladder, periodontal ligament, and neuronal models. Published work from our lab has shown that our 3-D models respond to infection with bacterial and viral pathogens in ways that reflect the infection process *in vivo*. By virtue of their physiological relevance, 3-D cell cultures may also hold significant potential as models to provide insight into the neuropathogenesis of HIV infection. Furthermore, the experimental flexibility, reproducibility, cost-efficiency, and high-

throughput platform afforded by these 3-D models may have important implications for the design and development of drugs with which to effectively treat neurological complications of HIV infection.

Keywords: 3-D cell culture, physiologically relevant, host-pathogen interactions, NeuroAIDS

Introduction

The more closely the phenotype of a cell culture model resembles its parental tissue, the greater will be the relevance of that model to the infection process *in vivo*. Thus, a more complete understanding of the mechanisms of human infectious disease will depend upon the development of physiologically relevant (i.e. organotypic) models of human cell cultures that can be used to identify and characterize host and microbial products important for infection. Such models also have exciting potential for the discovery and development of novel therapeutics.

Much of our knowledge of how microbial pathogens cause infection is based on studying experimental infections of standard monolayers grown as flat two-dimensional (2-D) cultures on impermeable surfaces of plastic or glass. While these models continue to contribute to our understanding of infectious diseases, they are greatly limited in the extent to which they model the complexity of an

intact 3-D tissue. In particular, this method of culture prevents the cells from establishing the 3-D architecture that is critical for the differentiated structure and function of parental tissues *in vivo* (Abbott, 2003; O'Brien et al, 2002; Schmeichel and Bissell, 2003; Zhang, 2004). In addition, cells grown as standard 2-D monolayers are also unable to respond to chemical and molecular gradients in three-dimensions (at apical, basal, and lateral cell surfaces) resulting in many departures from *in vivo* behavior (Zhang, 2004).

There are a variety of methods that have been used to enhance the differentiation of cultured cells, including permeable inserts, transplanted human cells grown as xenografts in animals, and explanted human biopsies. While these advanced *in vitro* models have provided important insights into microbial pathogenesis, they suffer from limitations such as short lifetimes, laborious set-up, experimental variability, availability and limited numbers of cells.

Use of the RWV bioreactor to engineer biologically meaningful 3-D cell cultures

Optimally, cell culture model design should mimic both the 3-D organization and differentiated function of an organ, while allowing for experimental analysis in a high throughput platform.

Originally designed by NASA engineers, the RWV is an optimized suspension culture technology for growing 3-D cells that maintain many of the specialized features of *in vivo* tissues (Nickerson et al, 2004; Unsworth and Lelkes, 1998). The RWV bioreactor is based on a rotating cylinder completely filled with culture medium, in which the sedimentation of cells within the vessel is offset by the rotating fluid, creating a constant fall of cells through the culture medium [**Figure 1**]. Oxygen is provided to cells through a hydrophobic membrane at the back of the bioreactor. These conditions maintain cells in suspension under levels of low, physiologically relevant fluid shear, enabling them to a) attach to one another and form aggregates based on natural cellular affinities, b) grow in three-dimensions, c) differentiate, and d) form the fragile connections that are required for complex tissue-like 3-D structures (Nickerson and Ott, 2004; Nickerson et al, 2004; Unsworth and Lelkes, 1998) [**Figure 1**].

Applying 3-D cell cultures to study infectious disease

Cell culture in the RWV is easy to perform. Cells are first grown as monolayers in tissue culture flasks. At the appropriate density, cells are removed from the flask, resuspended in medium, and incubated with porous *collagen-coated microcarrier beads for attachment [**Figure 1**] *(*note:* microcarriers can be coated with any extracellular matrix/ECM molecule of choice). The cell-bead complexes are then introduced into the RWV and rotation is initiated. 3-D cells cultured on

the surface of porous microcarrier beads are able to sense and respond to chemical and molecular gradients like the parental tissue *in vivo*. The medium is changed as necessary and vessel rotation speed is increased as cultures grow to maintain cells in suspension. The 3-D cell cultures are then removed from the bioreactor and distributed evenly in multi-well plates or other convenient formats for testing. 3-D cells are amenable to a wide range of experimental manipulations, and can be removed from the bead with various treatments for use in studies that require homogeneous cell suspensions, like flow cytometry.

Publications from our team have shown that a variety of different 3-D cell cultures, including human intestine, lung, and placenta models, more closely resemble the physiology of their parental tissues *in vivo* as compared to the same cells grown as monolayers, and respond to infection with microbial pathogens in ways that reflect the natural infection process (Carterson et al, 2005; Lamarca et al, 2005; Nickerson et al, 2001; Nickerson et al, 2004; Höner zu Bentrup et al., 2006). We reported the first use of 3-D cell culture models of human intestinal epithelium generated in the RWV to study the enteric pathogen *Salmonella typhimurium* (Nickerson et al, 2001) **[Figure 2]**. Compared to monolayers, 3-D culture of human intestinal cell lines (small intestine and colon) enhanced many characteristics associated with fully differentiated functional intestinal epithelia *in vivo*, including distinct apical and basolateral polarity, increased expression and better organization of tight junction, extracellular

matrix, and brush border proteins, and highly localized expression of mucins **[Figure 3]**. All of these important physiological features of *in vivo* intestinal epithelium were either absent or not expressed or distributed at physiologically relevant levels in monolayer cultures of these same cells (Nickerson et al, 2001; Höner zu Bentrup et al., 2006). Moreover, our 3-D intestinal cultures in the RWV grew predominantly as unilayers on porous collagen I coated microcarrier beads, which recapitulates normal intestinal epithelium *in vivo*, which consists of a single layer of cells **[Figure 4]**. This physiologically relevant pattern of growth was not observed for monolayers of the same cells, which grew past confluence and stacked on top of each other. When applied to study aspects of human enteric salmonellosis, our 3-D intestinal cultures responded in ways that were similar to an *in vivo* infection, including differences in tissue pathology, adherence, invasion, apoptosis, and cytokine expression (Nickerson et al, 2001; Nickerson et al, 2004; Höner zu Bentrup et al., 2006). Other published and/or submitted studies with these models from our lab include a) evaluation of known pathogenesis determinants of *Salmonella* (Honer zu Bentrup et al, 2006), b) evaluation of other enteric pathogens, including those difficult or previously not possible to cultivate in vitro (Straub et al, Submitted), and c) introduction of biological signals that mimic what such tissues encounter *in vivo*, as part of a broader effort to understand particular interactions between pathogens and the host intestinal epithelium.

Recently, we described the establishment and characterization of a 3-D model of human lung epithelium and its application to study the pathogenesis of *Pseudomonas aeruginosa* (Carterson et al, 2005). As with our 3-D intestinal models, cultivation of a human lung cell line in the RWV resulted in the formation of 3-D tissue aggregates that displayed important structural and functional characteristics of the differentiated parental tissue, including enhanced and extensive formation of tight junctions, extracellular matrix proteins, and mucus production. These features were not observed in monolayers of the same cells. In response to infection with *P. aeruginosa*, the 3-D lung cells, but not monolayers, responded in ways that were relevant to the infection *in vivo*, including differences in bacterial colonization, cellular morphology, and cytokine expression profiles. Collectively, these studies show that the use of the RWV to generate 3-D cultures from a variety of cell types has wide applications in the modeling of infectious disease.

Conclusions

3-D cell culture has wide applications for infectious disease studies and drug development. Indeed, 3-D cell cultures provide alternative organotypic models that will complement existing experimental systems to study human infectious disease, and are a powerful tool for the commercial development of novel therapeutics. We, along with our collaborators, have generated a variety of 3-D cell cultures from different human tissues that model many aspects of their *in*

vivo parental tissues, and which are currently being used in infection studies. These 3-D cell cultures include models of colon, lung, placenta, bladder, periodontal ligament, and neuronal cultures. By virtue of their physiological relevance, 3-D cell cultures may also hold significant potential as models to provide insight into the neuropathogenesis of HIV infection. For example, observations from preliminary studies show that our 3-D neuronal cultures exhibit morphological and functional characteristics relevant to their parental cells in vivo. Such models may hold important utility for NeuroAIDS research, including a) cross-clade assessment of infection in 3-D tissues examining HIV-1 variants, neurotoxic effects, and other host responses, b) the role of viral proteins and drugs in neurotoxicity, and c) the efficacy of candidate drugs. Furthermore, the experimental flexibility, reproducibility, cost-efficiency, and high-throughput platform afforded by these 3-D models may have important implications for the design and development of drugs to effectively treat neurological complications of HIV infection.

Beyond our infectious disease applications, 3-D cell cultures have been used in a variety of other biomedical applications such as cancer biology, stem cell research, the study of immune-cell interactions, the growth of tissues for transplantation, and the development and testing of novel therapeutics [http://www.synthecon.com].

These 3-D models are simple, high-throughput systems that create very large numbers of cells per experiment, and can be studied by techniques not possible or limited in many other advanced *in vitro* models. The high fidelity, reproducibility, and cost-efficiency of 3-D cell culture offers a powerful screening tool for therapeutics and holds tremendous potential for drug and target validation and discovery. 3-D cell-based models have been effectively used to re-examine molecular pathways previously characterized by conventional culture methodologies as well as to elucidate novel signaling pathways during normal cellular differentiation and tumor progression (Schmeichel and Bissell, 2003). By analogy, 3-D cell culture holds enormous potential for novel product development for the diagnosis, prevention, and treatment of infectious disease.

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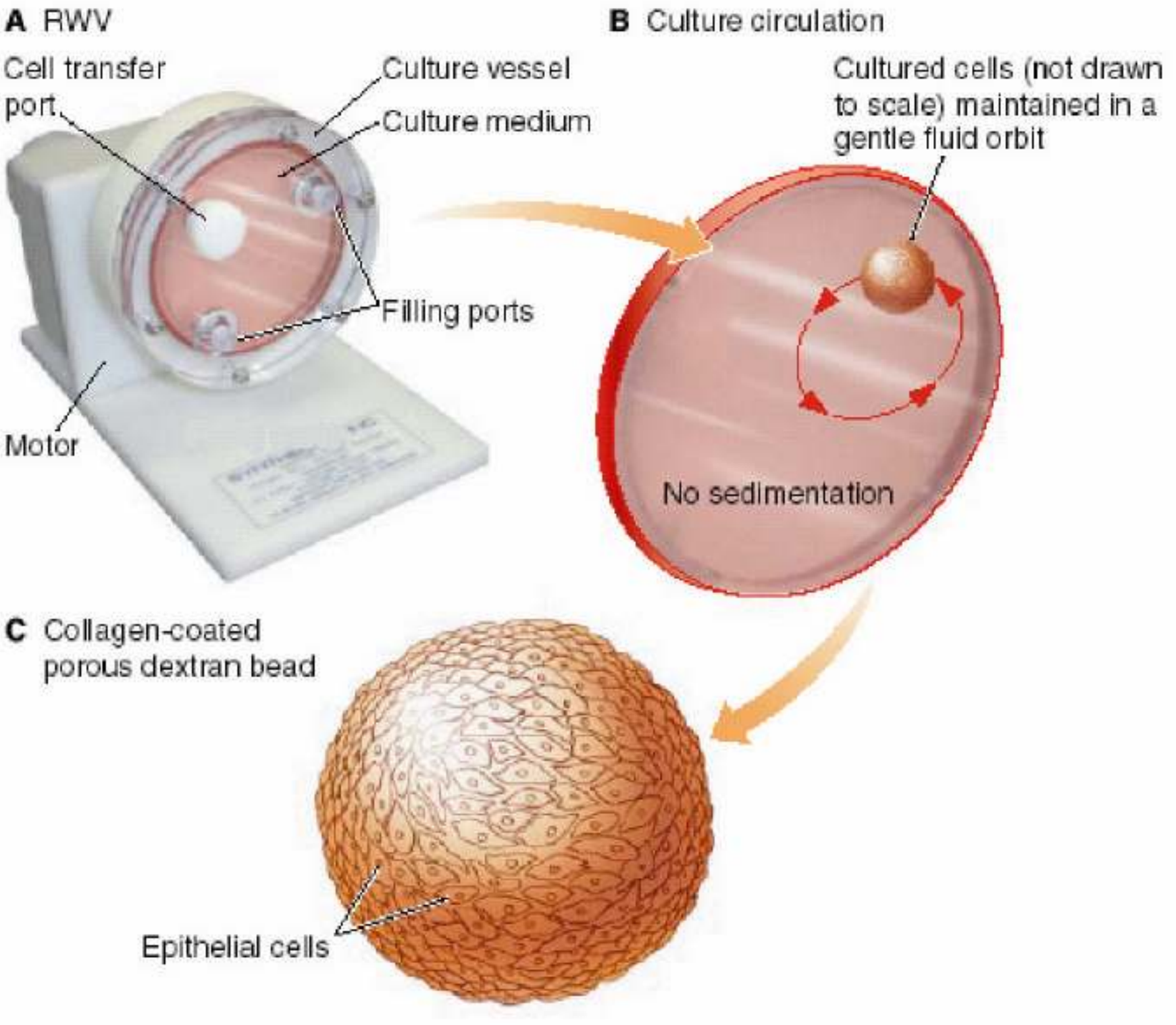
Figure 1: (A) **Operation of the RWV**. The vessel is filled with growth medium and cells are added. Vessel rotation is initiated. (B) Cells cultured in the RWV are maintained in a gentle fluid orbit. (C) Depiction of a collagen-coated microcarrier bead to which cells have attached on the surface. Reprinted with permission from the American Society for Microbiology (ASM News, April 2004, p.169-175).

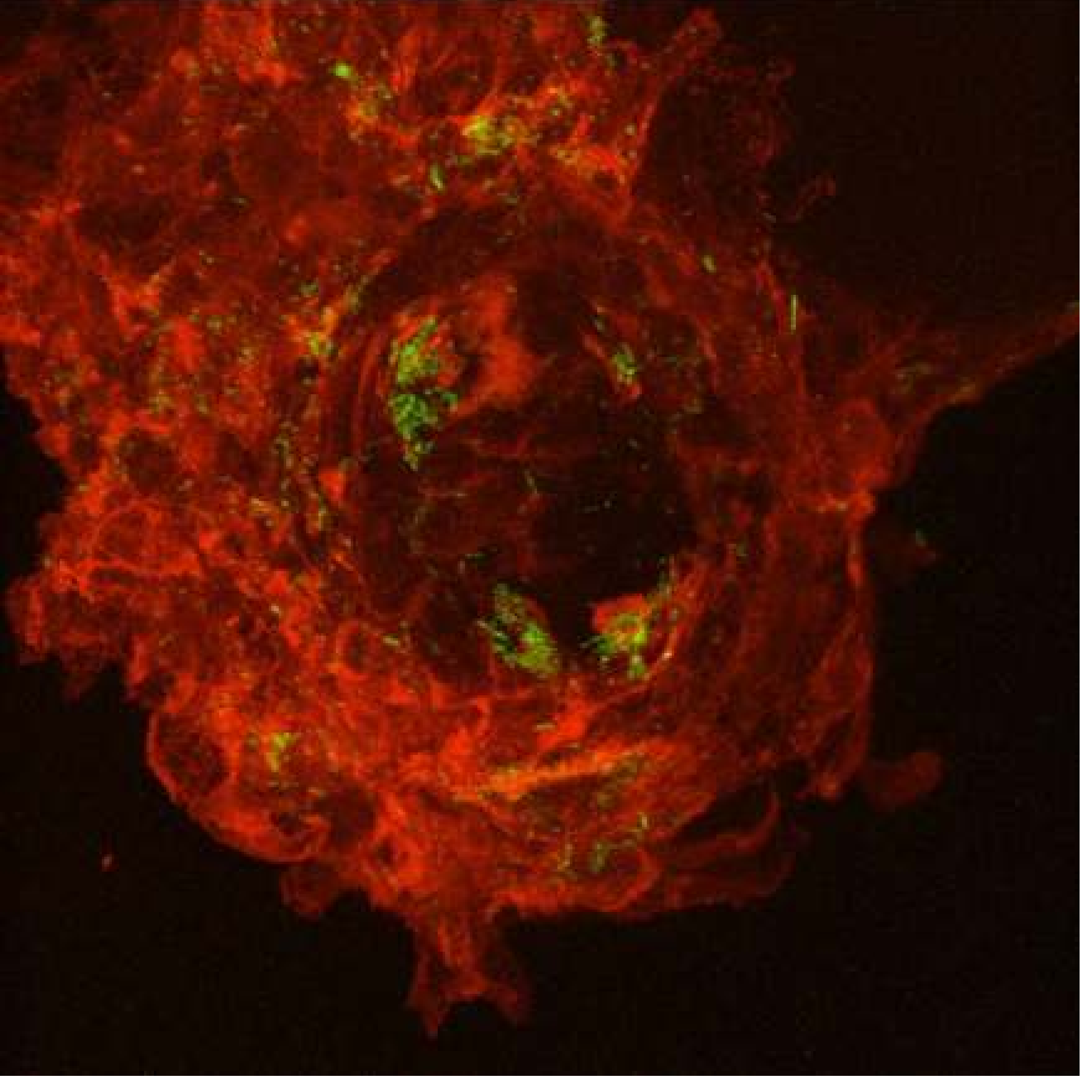
Figure 2: Confocal image of 3-D model small intestinal epithelium (Int-407 cells) following infection with *S. typhimurium*. Phalloidin labeling of the actin cytoskeleton of 3-D Int-407 cells (red) after infection with GFP-labeled *S. typhimurium* (green). Reprinted with permission from the American Society for Microbiology (ASM News, April 2004, p.169-175).

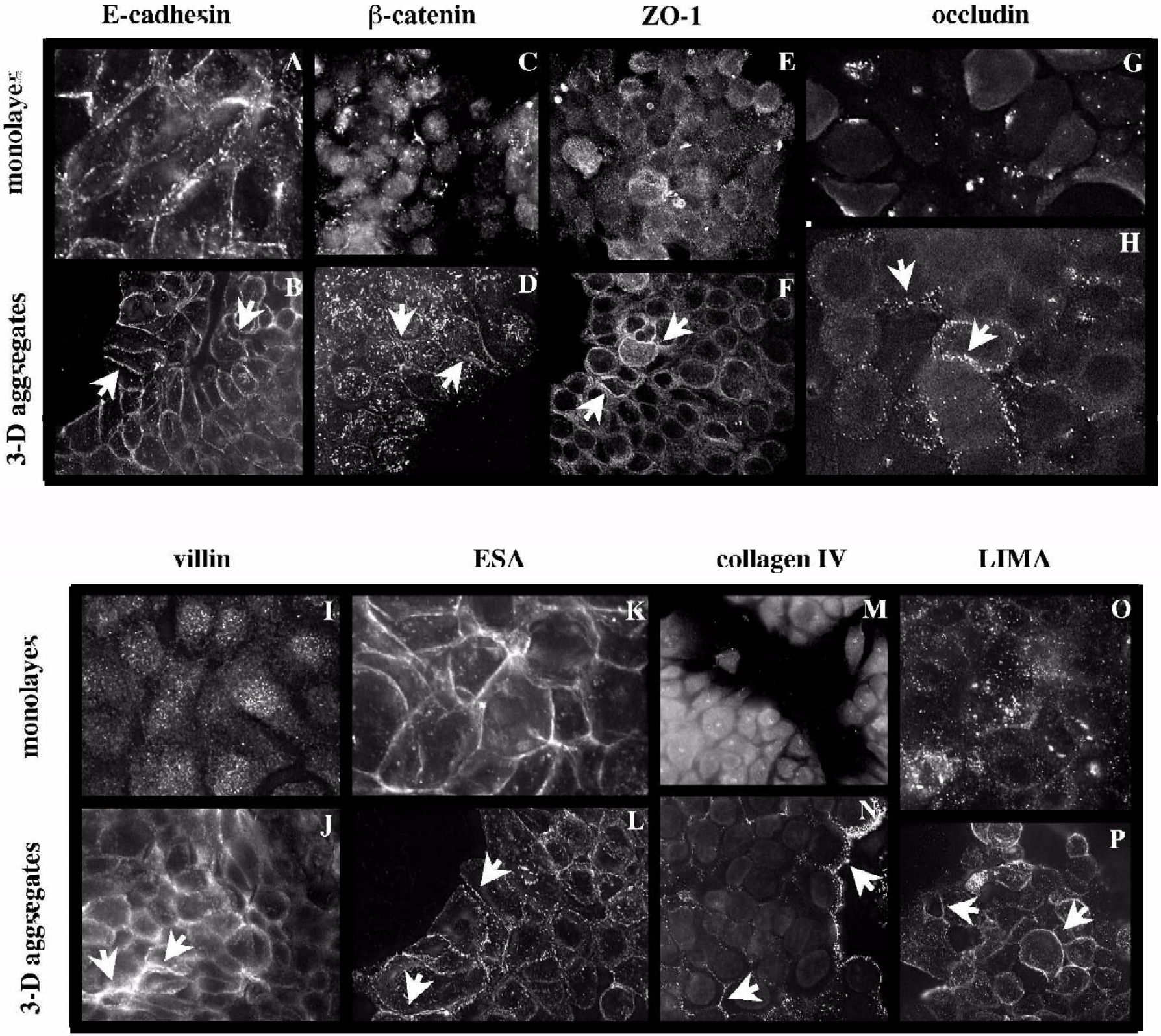
Figure 3: Immunohistochemical profiling of HT-29 colon cells grown as monolayers (A, C, E, G, I, K, M, and O) or as 3-D aggregates (B, D, F, H, J, L, N, and P). Epifluorescent images of HT-29 cultures stained with antibodies against junctional proteins E-cadherin (A, B), β-catenin (C, D), ZO-1 (E, F), and occludin (G, H), villin, a marker for brush border differentiation (I, J), epithelial specific antigen (ESA; K, L), type 4 collagen, an ECM-marker (M, N) and large intestinal mucinous antigen (LIMA; O, P). Images represent a merging of sections spanning the apical/lateral region of HT-29 cells (for junctional markers, LIMA, villin) or the basolateral region (for type 4 collagen, ESA). Arrows point to

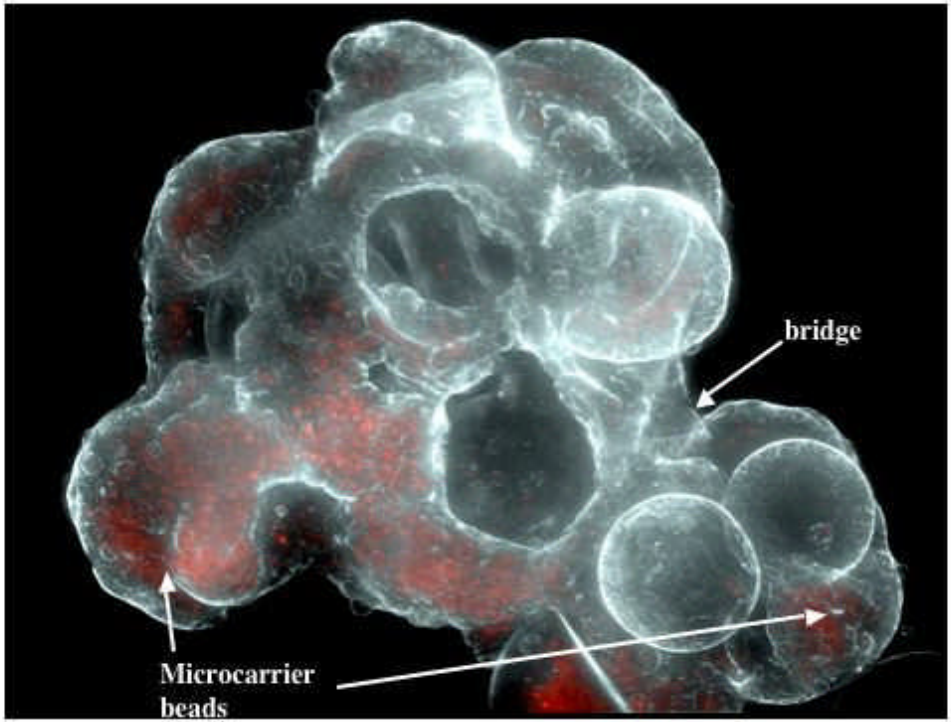
examples of major differences in expression and distribution patterns of these proteins between 3-D aggregates and monolayers. (Magnification: 630 X, with additional 2X optical magnification for panels A,G, H, and K). Data are from a single experiment and are representative of three separate experiments, from independent batches of cells. (Reprinted with permission from Microbes and Infection, Höner zu Bentrup, et al., 2006, In Press).

Figure 4: HT-29 colon cells form unilayers on microcarrier beads when grown in 3-D. (A) Double immunostaining to show that HT-29 cells grown in the RWV grow predominantly as unilayers on porous collagen I coated microcarrier beads, which is relevant to the parental tissue *in vivo*. Cell walls are stained with ESA (pseudocoloured gray) while nuclei are counterstained with DAPI (pseudocoloured red). Image obtained by widefield deconvolution microscopy midway through a typical aggregate. Magnification 100 X. Arrows point to unilayers of cells on the surface of microcarrier beads as well as multilayered bridges linking these regions (Reprinted with permission from Microbes and Infection, Höner zu Bentrup et al., 2006).


A RWV
Cell transfer port
Culture vessel
Culture medium
Filling ports
Motor
B Culture circulation
Cultured cells (not drawn to scale) maintained in a gentle fluid orbit
No sedimentation
C Collagen-coated porous dextran bead
Epithelial cells




E-cadhesin
β-catenin
ZO-1
occludin
monolayer
3-D aggregates
A
B
C
D
E
F
G
H
villin
ESA
collagen IV
LIMA
monolayer
3-D aggregates
I
J
K
L
M
N
O
P


bridge
Microcarrier
beads