



**PELO**Biotech *Competence<sup>4</sup> Cells*

## 3D Cell Culture

Cells

Media

Proteins

Tools



**We offer Technologies for  
3D Cell Culture and  
3D Models ready-to-assay**

(Nov. 15)

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## Our Partners and Network for 3D Cell Culture



## Rely on & Relax

### Welcome to your One-Stop-Cell Culture Shop,

Being competent<sup>4</sup> cells – that is PELOBiotech's outstanding characteristic. We want you to get the best and reproducible results ever, results you can rely on and relax. We offer special cell culture media for human primary cells, stem cells and embryonic stem cells as well as special supplements for tumor stem cells. We get you the whole variety of solutions from tissue dissociation to cryopreservation.

Our mission is to provide high quality cell culture products at affordable prices. Therefore we are at the top-level of international research regarding consistent quality. We block out the Black Box esp. while developing Defined or Xeno-free Media limiting usage of FBS and other animal products. We have built up a worldwide network of competent and innovative partners. Our network clears your direct access to products of the future research.

We offer different 3D Technologies:

- Scaffold-free
  - Magnetic Levitation
  - 3D Bioprinting
- Scaffold-based
  - Hyaluronic Acid Scaffolds
  - Alvetex® Scaffolds
  - Electrospun Nanofiber Scaffolds
- Hydrogel-based
  - Collagen
  - Mimsys G
- 3D Models Ready-to-Use
  - 3D Liver Microtissues
  - 3D Tumor Microtissues
  - 3D Pancreas Microtissue
  - 3D Skin Model
  - 3D Airway Model

Contact us today and let us show you how we can help you meet your technical and budget targets and bring your research from bench to bedside.

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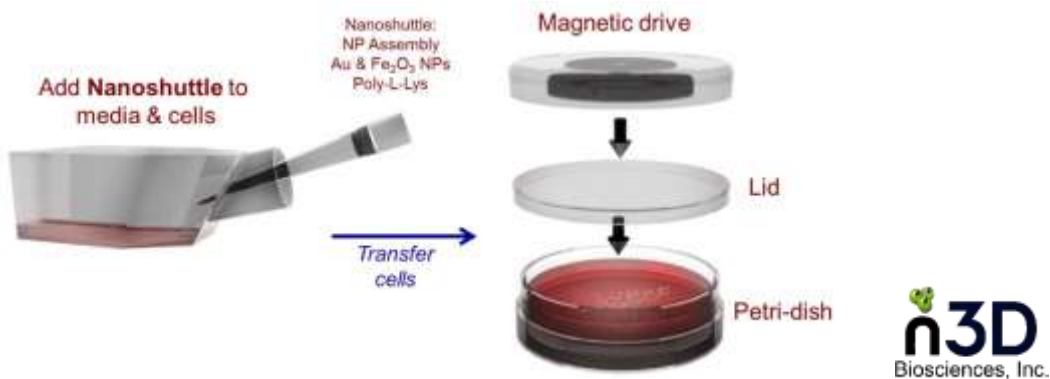
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## 3D - SCAFFOLD-free

### Magnetic Levitation

Our partner Nano 3D Biosciences has developed a propriety technology using magnetic forces to produce 3D spheroids in different formats. In magnetic levitation, cells are magnetized with NanoShuttle™-PL through overnight incubation and dispensed into a ultra low-attachment multiwell plate, where they are levitated off the bottom by a magnet above the plate. The magnetic forces work as an invisible scaffold that rapidly aggregates cells, and induces cell-cell interactions and ECM synthesis. The 3D culture is formed without any artificial substrate or specialized media or equipment and can be cultured long-term.



The heart of n3D's cell culturing technology, NanoShuttle™-PL consists of gold, iron oxide, and poly-L-lysine. NanoShuttle™-PL magnetizes cells by electrostatically attaching to cell membranes during an overnight static incubation. Magnetized cells will appear peppered with dark nanoparticles after incubation. NanoShuttle™-PL will stay attached to the cell membrane for up to 8 days, at which point it's released into the 3D culture. NanoShuttle™-PL is biocompatible, having no effect on metabolism, proliferation, and inflammatory stress, and even encouraging proliferation in 3D. Additionally, it does not interfere with other experimental techniques, such as fluorescence, or Western blotting.



Fig. (Source: n3D): Bio-Assembler I from 1-Well to 24 Well format.



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## 3D - SCAFFOLD-free

### Magnetic 3D Bioprinting in 96-well and 384-well format

**What is 3D bioprinting ?** It magnetize cells with NanoShuttle (NS) to print spheroids or cellular rings. In contrast to magnetic levitation using the Bio-Assembler, in magnetic 3D bioprinting, cells incubated with NS overnight are printed into spheroids by placing a 96-well plate or 384-well plate full of magnetized cells atop a drive of magnets. The magnets below the well aggregate the cells using mild magnetic forces to form a spheroid or rings at the bottom of the well. After only 15 min to a few hours, the plate of spheroids can be removed from the magnets and cultured long-term.

**Advantage:** Adding and removing solutions is made easy by the use of magnetic forces to hold down spheroids during aspiration, limiting spheroid loss. Spheroids can also be picked up and transferred between vessels using magnetic tools such as the MagPen™.

#### Magnetic 3D Bioprinting Shapes:

##### Ring Pattern



##### Dot or Spheroid Pattern



← Contraction Toxicity or Drug Effect Dilation →



Immediately after printing, these structures will shrink/close, as a function of cell migration, viability, and proliferation, and varies with dosage. Shrinkage is captured using a compact imaging kit (n3Dock) with an iPod™ programmed by a freely available app (Experimental Assistant) to image whole plates at specific intervals, forgoing the need to image well-by-well under a microscope. Shrinkage is complete within a day, and images are batch processed to rapidly yield toxicity data. Moreover, as shrinkage is label-free, the remaining rings or spheroids are available for further experimentation (IHC, Western blot, genomics, etc.).

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## 3D - SCAFFOLD-based

### Hyaluronic Acid Scaffolds



Our partner Celenys has developed Biomimesys<sup>®</sup> an innovative hydrogel for 3D cell culture. It is based on hyaluronic acid (HA), which is the main component of the extracellular matrix (ECM).

Biomimesys<sup>®</sup> is highly porous (150-200  $\mu\text{m}$ ) and creates a scaffold of reticulated Hyaluronic Acid (HA) chains for 3D cell culture providing a physiological environment much closer to the biological reality.

Biomimesys<sup>®</sup> is provided in a ready-to-use 96-well plate with a hydrogel into each well. Cells are simply seeded on top of the hydrogel using the cell culture medium of your choice. Incubation is carried out in normal conditions. The media can be refreshed easily by pipetting.

#### What can I do with Biomimesys<sup>®</sup> ?

It gives a more predictive tool in various applications: drug development, toxicity assessment, cell-based assays in many different research areas.

**Application:** So far Celenys tested primary cells (Fibroblast), Cancer Cell Lines, Cell Lines, Adipocytes, Hepatocytes and stem cells.

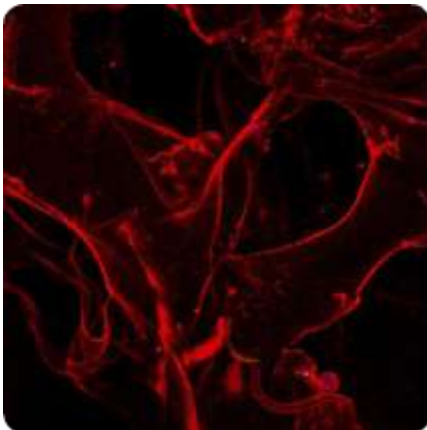


Fig. 1: HA chains structure visualized by LSCM

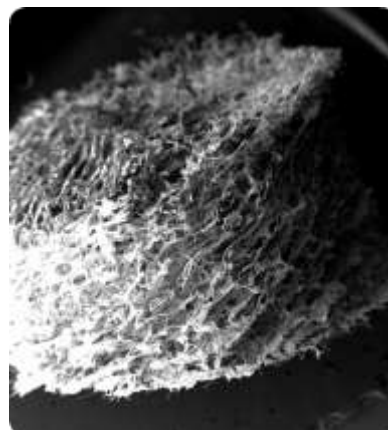


Fig. 2: Hydrogel architecture visualized by SEM



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(Source: Celenys)



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## 3D - SCAFFOLD-based

### Hyaluronic Acid Scaffolds



Our partner Celenys offers Biomimesys® for 3D adipocyte culture. It is based on hyaluronic acid (HA), which comes with some modifications to mimic a good environment for adipocytes.

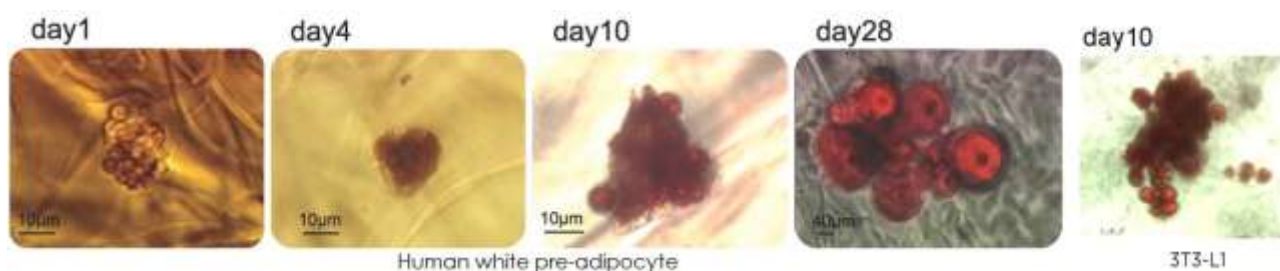


Fig.(Source: Celenys): At 10 days of nutrition, adipocytes in 3D are fully differentiated (oil red) compared with 2D, where undifferentiated cells are always present.

Further products are under development and will be launched soon.

**Advantage:** Biomimesys® is easy-to-use. You need only to seed cells on top of the scaffold and after 1 hour you can add already your culture medium.

Biomimesys® has many properties that make it ideal for use with numerous downstream applications. It is transparent for direct microscopy visualisation. The growing cells are easily retrieved from the matrix by a gentle and rapid procedure, this means that cells cultured in Biomimesys® may be analysed using all technologies as shown below.

- Brightfield Microscopy
- Fluorescence Microscopy
- Scanning Electron Microscopy
- Plate Readers/Spectrophotometer
- Flow Cytometry
- RNA or DNA Extraction
- Protein Extraction



Biomimesys® for Hepatocytes is available!

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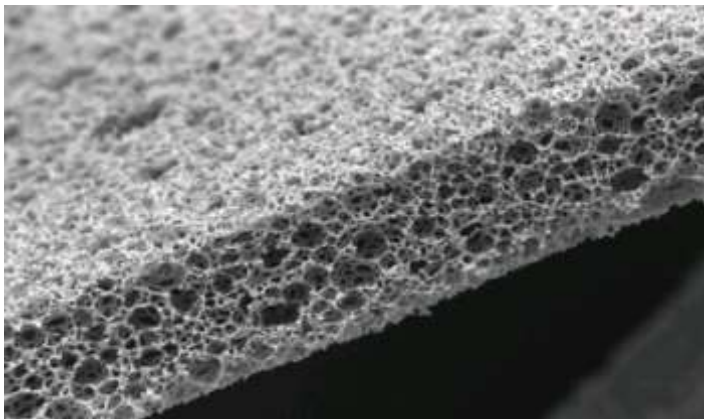
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Your ONE-STOP-CELL CULTURE SHOP

## 3D - SCAFFOLD-based

### Alvetex®

**What is Alvetex®?** It is a highly porous polystyrene scaffold designed for 3D cell culture. Cells grown in Alvetex possess a natural tissue-like structure that enables them to function in a more physiologically relevant manner. Alvetex 3D cell culture enables cells maintain their in vivo morphology, behavior and responsiveness within an in vitro model system.



#### **Alvetex® is a unique cellular environment**

(Source: ReprocellGroup)

Manufactured to the highest standards of consistency each disc is engineered to a thickness of just 200 microns with pore sizes of 36-40 microns. No cell is ever further than 100 microns from the nutrient source enabling easily exchange of nutrients, gases and waste products by passive diffusion across short distances.

Say goodbye to risks of foreign unknown materials such as proteins or cytokines of animal origin – unlike conventional materials used in cell culture Alvetex® is made from polystyrene and is completely inert.

**Advantage:** Alvetex® has been designed for simple and routine use. It uses conventional cell culture plasticware. Therefore any cell biologist can get into Alvetex 3D cell culture.



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## 3D - SCAFFOLD-based

### Alvetex®

It is easy to use!

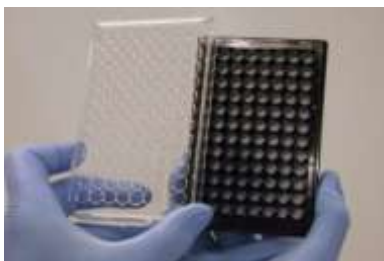
Just unwrap product, prepare scaffold, add cell suspension in appropriate medium at optimized seeding density and place in incubator to culture cells in 3D

#### Is Alvetex® 3D cell culture compatible with other assays?

In vitro derived 3D tissues grown on Alvetex can be studied using a variety of standard molecular and cellular techniques:

- Tissue processing, fixation, embedding and sectioning
- Histological staining, in situ hybridisation
- Bright-field microscopy and photographic imaging
- Electron microscopy – both SEM and TEM
- Cryostat sectioning
- Immunocytochemistry
- Fluorescence microscopy, confocal, laser capture
- Isolation of viable cells for passaging
- Flow cytometry and cytospinning
- Extraction of nucleic acid and total protein
- Biochemical assays

**Application:** A huge number of applications already exist. Just check our [www.reinnervate.com](http://www.reinnervate.com) for further information.



(Source: ReproCellGroup)

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## 3D - SCAFFOLD-based

### Electrospun Nanofiber Scaffolds

**What is AK-PolyFibers?** It is Akron Biotech's new range of 3D scaffolds made from electrospun polymeric nanofibers. From 3D cell culture scaffolds that offer cells an environment that closely resembles the mechanical and physical features of natural ECM to custom 3D tissue engineered constructs, AK-PolyFibers are innovative, versatile and robust.

**Advantage:**

- Multiple configurations: Plate inserts and custom 3D-engineered constructs
- Create a more natural environment for your cells
- Controlled cell expansion and differentiation
- Allows easy removal of cells for assays and gene expression
- Natural and synthetic polymers: PLA, PLGA, PCL, collagen, gelatin and more

AK PolyFibers are available as 6, 12 and 24 well plate inserts.

**Application:** Already used for 3D Cell Culture of mesenchymal stem cells, cord blood cells, bone marrow cells and breast cancer cells.

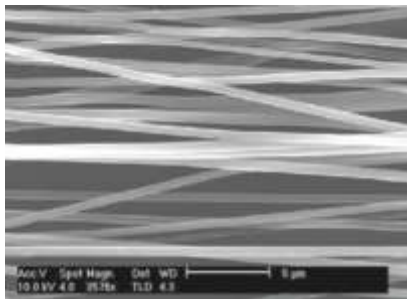


Fig 1: Aligned AK-PolyFibers

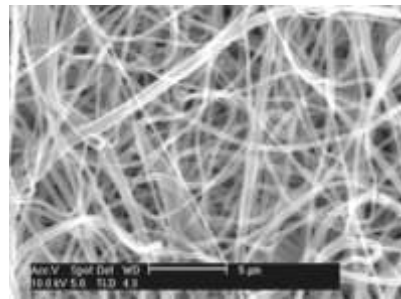


Fig. 2: Random AK-PolyFibers

(Source: Akron Biotech)



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## 3D – Hydrogel-based

### Collagen based / Human / Rat / Bovine

**What is CollaGel?** This Hydrogel can be used as two- or three-dimensional scaffolds for cells; it mimics the natural environment of cells, both chemically and physically.

**Advantage:** CollaGel Hydrogel matches the softness of the body's tissues.

**Application:** It can be used to culture neurons in an environment as soft as the brain, cardiomyocytes on the stiffness of muscle, or whatever your cells' tissue of origin may be. In a 3D tissue model, our CollaGel Hydrogel helps the cells build stronger basement membranes.

### mimsys G

Cat#: PB-060201-1 (1 g) - PB-060201-02 (0,2 g)



**What is mimsys G?** It allows formation of hydrogel in the presence of monovalent cations or by addition of photo-initiator and ultraviolet light.

mimsys G forms biocompatible hydrogels capable of chemical manipulation for use either as acellular or cellular systems, dispensed manually or automatically at the application site. Its hydrogels are suitable for incorporation of cells, biomolecules, therapeutic drugs, diagnostic markers, probes, and/or other agents useful for tissue engineering and regenerative medicine applications<sup>4,5</sup>, as well as for their controlled delivery/release. mimsys G can be processed into different types of scaffolds, such as hydrogels, fibres, 3D structures and micro- or nanoparticles<sup>3</sup>.

3. J. Silva-Correia, J.M. Oliveira, J.T. Oliveira, R.A. Sousa, R.L. Reis, Photo-crosslinked gellan gum-based hydrogels: methods and uses thereof. WO2011/119059, Priority date: 105030 26.03.2010 PT.

4. J. Silva-Correia, J.M. Oliveira, S.G. Caridade, J.T. Oliveria, R.A. Sousa, J.F. Mano, R.L. Reis, Gellan gum-based hydrogels for intervertebral disc tissue-engineering applications. J Tissue Eng Regen Med, 2011. 5(6): p. e97-e107.

5. J. Silva-Correia, V. Miranda-Gonçalves, A. Salgado, N. Sousa, J.M. Oliveira, R.M. Reis, R.L. Reis, Angiogenic potential of gellan gum-based hydrogels for application in nucleus pulposus regeneration: In vivo study, Tissue Eng A, 2012. (doi:10.1089/ten.TEA.2011.0632)



mimsys G main repeat unit  
(Source: irisbiosciences)



Check out the MicroMatrix Arrays to find the optimal Microenvironment for your cells.

## 3D – Models ready-to-use

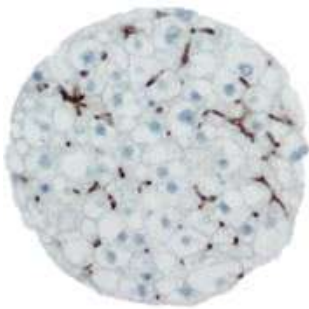
### 3D InSight™ Liver Microtissues for Toxicology

#### What is 3D InSight™ Human Liver Microtissue?

It is generated by co-culture of cryopreserved primary human hepatocytes and non-parenchymal cells (NPCs) to reflect the native cell composition of liver.

Available are:

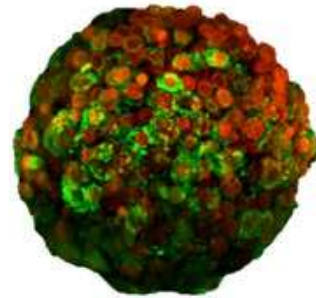
- Human Liver Microtissues
- Rat Liver Microtissues
- HepG2 Liver Microtissues



Primary human  
cryopreserved  
hepatocytes +/- NPCs



Source: Fresh rat liver  
+/- NPC



Source: ATCC,  
human hepatoma

### 3D InSight™ Liver Toxicology Service Packs

for more information please contact us



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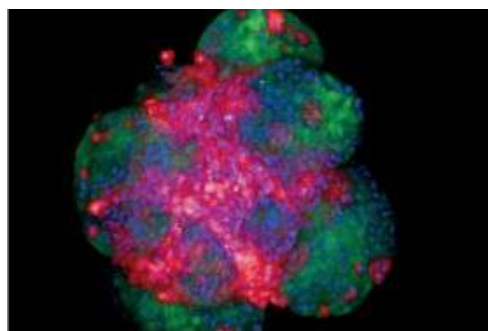
## 3D - Models ready-to-use

### 3D InSight™ Tumor Microtissues for Oncology

**What are cancer microtissues?** InSphero's microtissues exhibit morphological and functional characteristics that correspond to the inter-vascular regions of solid tumors or avascular micrometastasis, in other words cell-cell contacts, cell-cell communication, cell type-specific extracellular matrix, cell-matrix interaction and pathophysiological conditions.

**Advantage:**

- 100's of models available based on widely used cell lines
- Various co-culture options using mouse or human stromal cells
- 3D InSight™ Microtissues for in-house use and fee-for-service offers available
- Customer-specific model development with turnaround times of 6 weeks



**Application:** Cancer Microtissues are available as homotypic and heterotypic spheroids. Heterotypic microtissues (co-cultures) are available with NIH-3T3 cells (mouse fibroblast cell line) or NHDF (normal human dermal fibroblasts). Available are microtissues of the following tissues:

Lung	Prostate	Kidney
Liver	Glioblastoma	Osteosarcoma
Colorectal	Ovarian	
Breast	Cervical	



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## 3D - Models ready-to-use

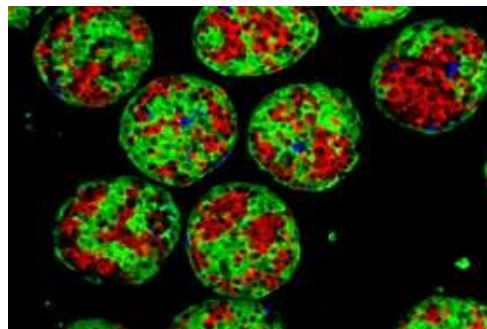
### 3D InSight™ Human Pancreatic Microislets

**What are Primary Human Pancreatic Islets?** They are of great importance for in vitro research related to diabetes and metabolic disorders. The purity and viability of pancreatic islets can vary significantly between different isolations depending on donor characteristics, cause of death and other confounding factors. Exocrine tissue impurities, size heterogeneity and partial cell death are the main factors which negatively affect data robustness obtained from experiments using freshly isolated pancreatic islets.

**Advantage:** InSphero's patented microtissue technology produces islets of increased purity, viability and size homogeneity, and delivers one microtissue per well in our 96-well GravityTRAP™ plate. The process increases throughput by eliminating tedious hand-picking of similarly sized islets and separation from exocrine tissue, while providing tissues in an assay-ready, automation-compatible format.

#### **Long-lived, standardized pancreatic islet microtissues for diabetes & metabolic research**

- Viability and glucose-stimulated insulin secretion persist for >4 weeks
- Homogeneous microislet size eliminates tedious hand-picking
- Uniform ratio of alpha, beta, and delta cells minimizes islet-to-islet variation
- Delivered in 96-well non-adherent GravityTRAP™ plates





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## 3D - Models ready-to-use

### 3D Skin Model

**What is CellApplications' 3D Skin Model?** It is a highly physiological, three-dimensional cellular system of Human Epidermal Keratinocytes (HEK) for in vitro studies, offering an excellent cellular system to examine aspects of epithelial function and disease, particularly those related to skin biology and toxicology.

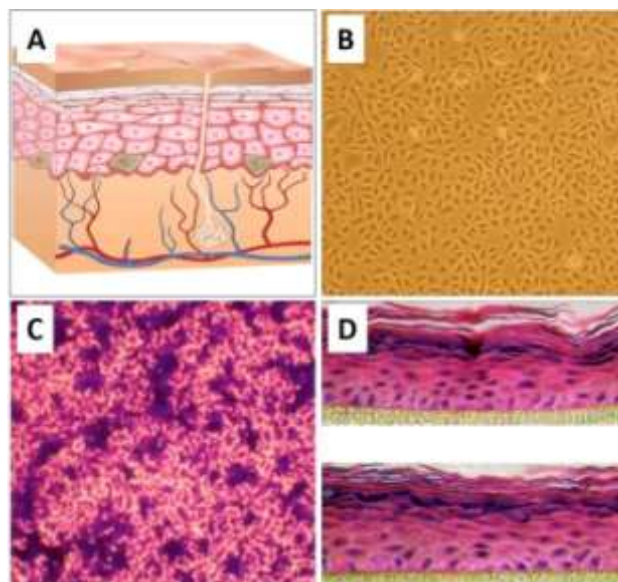
**Advantage:** Basic science and pharmaceutical researchers use the 3D Skin Model to replace animal tests for the assessment of skin irritation due to chemicals, skin-cream-based drugs and cosmetics.

**Application:** CAI's Skin Model can also help avoid misclassification of chemicals and skin corrosion observed in animal systems (1, 2). Other applications include phototoxicity, percutaneous absorption & penetration, wound healing and metabolism. The cells are grown and differentiated into a stratified squamous epithelium on PCF inserts with a liquid/air interface. Contact us for more information.

1) Eun & Nam, *Exog Dermatol*, 2:1–5 (2003)

2) York et al., *Contact Dermatitis*, 34:204 (1996)

A. Depiction of Human Skin. B. Human Epidermal Keratinocytes (HEK) at ~80% confluency. C. Crystal Violet Staining. D. Hematoxylin & Eosin Staining of HEK differentiated into stratified squamous epithelium after 14 days.



The 3-D skin model is available as 12 or 24 well do-it-yourself-kit. The cells will be delivered cryopreserved.

Source: CellApplications.

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## 3D - Models ready-to-use

### 3D Airway Model

**What is the 3D Airway Model?** It is a highly physiological, three-dimensional cellular system of Human Bronchial Epithelial Cells (HBEpC).

**Application:** It is used for in vitro examination of epithelial function and disease, including airway infections, tissue repair mechanisms, signaling changes and potential treatments relevant to lung injuries, mechanical and oxidative stress, inflammation, pulmonary diseases and smoking. The cells are grown and differentiated into a pseudostratified epithelium on PCF inserts with a liquid/air interface.

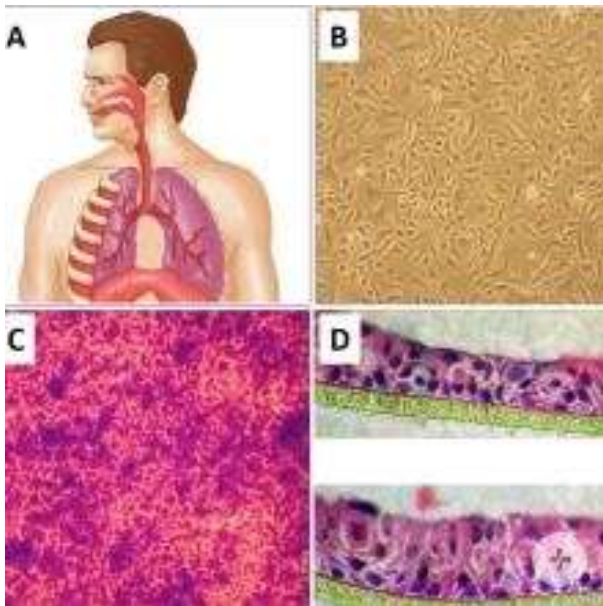


Fig A. Depiction of Human Airway.

Fig B. Human Bronchial Epithelial Cell (HBEpC).

Fig C. Crystal Violet Staining.

Fig D. Hematoxylin & Eosin Staining of HBEpC differentiated into pseudostratified epithelium after 28 days.

(Source: CellApplications)

The 3D airway model is available as 12 or 24 well do-it-yourself-kit. The cells will be delivered cryopreserved.



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