A scanning electron micrograph (SEM) showing a dense array of small, spherical platinum nanoparticles. The particles are arranged in a somewhat regular pattern, appearing as dark, circular spots against a lighter background. The overall image has a purple and grey color scheme.

# Cell and Tissue Engineering

▲ Tailored hollow spherical platinum nanoparticles with high catalytic activity in oxidation reactions.

**IBN**

**TECHNOLOGY PORTFOLIO**



## About IBN

The Institute of Bioengineering and Nanotechnology (IBN) is the world's first bioengineering and nanotechnology research institute. Since 2003, IBN has been conducting interdisciplinary research bridging science, engineering and medicine. The Institute's strengths lie in its synthetic capability for chemicals, materials and biologics. IBN has developed unique technology platforms that combine novel catalytic chemistry, biomaterials, nanofabricated devices and microfluidic systems with biological engineering. Its highly collaborative environment also promotes the sharing of ideas, expertise and infrastructural support, and a culture that encourages innovative research and the nurturing of young talents.

IBN's research activities are focused in the following areas:

**Drug and Gene Delivery**, where the controlled release of therapeutics involve the use of functionalized polymers, hydrogels and biologics for targeting diseased cells and organs, and for responding to specific biological stimuli.

**Cell and Tissue Engineering**, where biomimicking materials, stem cell technology, microfluidic systems and bioimaging tools are combined to develop novel approaches to regenerative medicine and artificial organs.

**Biodevices and Diagnostics**, which involve nanotechnology and microfabricated platforms for high-throughput biomarker and drug screening, automated biologics synthesis, and rapid disease diagnosis.

**Pharmaceuticals Synthesis and Green Chemistry**, which encompasses the efficient catalytic synthesis of chiral pharmaceuticals, and new nanocomposite materials for sustainable technology and alternative energy generation.

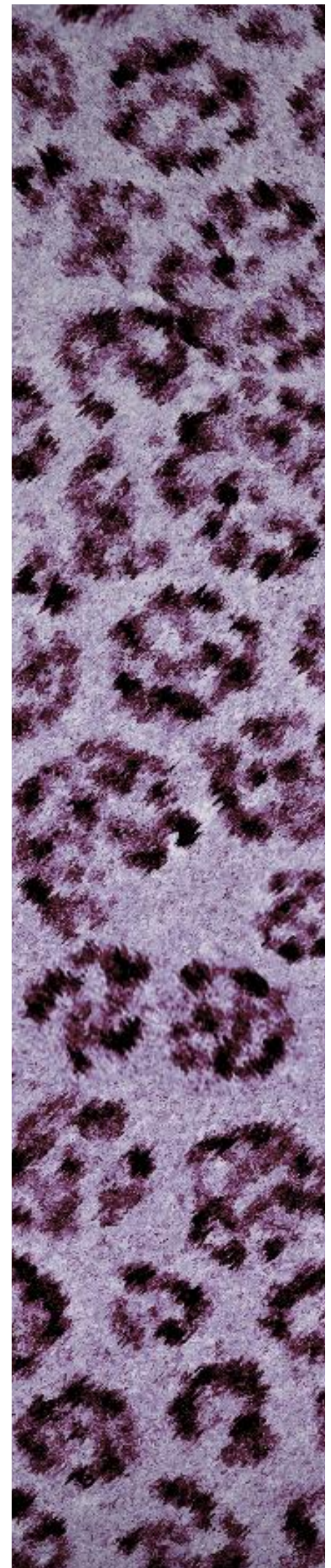
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As of February 2011, IBN has filed over 1,168 patent applications on its inventions and the Institute is currently looking for partners for collaboration and commercialization of its suite of technologies.

For more information, please visit [www.ibn.a-star.edu.sg](http://www.ibn.a-star.edu.sg) or contact Dr Benjamin Tai at [btai@ibn.a-star.edu.sg](mailto:btai@ibn.a-star.edu.sg) or +65 6824 7223.

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# Cell and Tissue Engineering



▲  
Thixotropic gel for 3D cell culture.

## A Mechanically Reversible Gel for Three-Dimensional Cell Culture and Tissue Engineering

The current practice of using petri-dishes dates back to the 1950s. Cells proliferating two-dimensionally on the surface of the culture media are an over-simplification of cell behavior in *in vivo* conditions especially for eukaryotic cells. This is because, with the exception of epithelial tissue, most other eukaryotic cells exist naturally embedded in a 3D extracellular matrix. For example, fibroblasts cultured in 2D show a high concentration of stress fibers on the surface of the cell that is in contact with the plate. Some technologies have been developed to construct a gel-like culture media that allow 3D growth of cells. These technologies, however, rely heavily on the use of chemical crosslinkers, photo-initiators, ultraviolet light or a change in ionic strength for cross-linking of gels. As a result, gelation has to be performed with extreme care and in the absence of cells and proteins. This makes it inconvenient for the manipulation of the media for cell work. Other protein-based gels rely on temperature to induce gelation. In addition to the inconvenience of usage, such gels also introduce inconsistencies in gelation time. At IBN, a novel polymer-silica composite gel has been developed that is suitable for both 3D cell culture and tissue engineering. It is liquefied by vortexing to allow for ligands and cells to be added. The gel resets when it is left to stand for a few minutes. Such reversible thixotropic properties are based on the dynamics of Van der Waals forces and thus do not require chemicals or heat.

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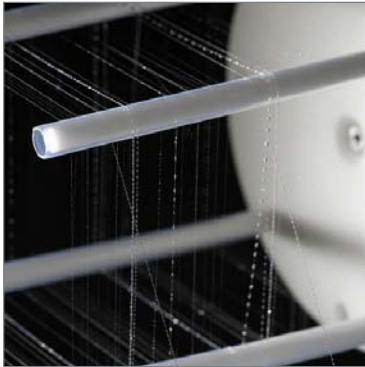
### Advantages:

- Convenient to use, as there is no need for chemicals or heat for gelation.
- Ease of cell harvesting, as there is no need for trypsination.
- Consistent gelation time, because the setting time of the gel is controlled by its composition, which is determined during the manufacturing process.
- Can be used with labware of different shapes and sizes and hence can be easily molded to fit the shape of the holding container.

### Applications:

- 3D cell culture for cell lines, primary cells or stem cells.
- Tissue culture as *in vitro* models for cancer research, drug development and cytotoxicity testing or high-content drug screening.
- *In vitro* tissue engineering and *in vivo* tissue/organ regeneration.
- Substrate for cell labeling and imaging.

# Cell and Tissue Engineering



▲ Process of drawing polyelectrolyte fibers.

## Fibrous Scaffolds and Extracellular Matrix Scaffolds for Tissue Engineering

Tissue engineering requires the use of a structural framework to support the adhesion and growth of cells. In addition to acting as a structural template, scaffolds provide the opportunity for the presentation of biological molecules that regulate the proliferation and differentiation of cells into tissue structures. This is especially important in the use of stem and progenitor cells. Most of the current processes for the production of tissue scaffolds are incompatible with the incorporation of biological molecules, specifically proteins, due to the severity of preparation conditions involving the use of high temperatures and organic solvents. At IBN, fibrous scaffolds are processed by interfacial polyelectrolyte complexation, which is inherently more suitable for the presentation of biologics due to the milder, aqueous-based fiber process. The characteristics of these fibrous scaffolds are optimized to potentiate the activity of various biological factors with respect to tissue development and stem cell expansion/differentiation. Methods such as hydroentanglement and fiber assembly are used to form non-woven and structured 3D scaffolds. Previous work in this area was hampered by the tendency of the fibers to clump together and form a dense monolith of scaffolds with low porosity, which has been solved by the incorporation of silica. Results have shown that cells could adhere and grow well within IBN's fibrous scaffolds after they were incorporated with components such as collagen and fibronectin. In addition, a novel method of reconstituting ECMs into fibrous scaffolds from both tissues and cell lines has been developed.

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### Advantages:

- Mild, aqueous-based fabrication process provides better conditions for cell culture as compared to conventional processes, and does not require high temperatures or the use of organic solvents.
- High surface-to-volume ratio of the resulting porous scaffolds provides a better environment for the culture of various cell types for tissue engineering.
- Ability to reconstitute ECMs from cell lines provides ease of availability and scale-up, and greater safety control.
- Ability to pattern cells in 3D improves tissue structure and function.

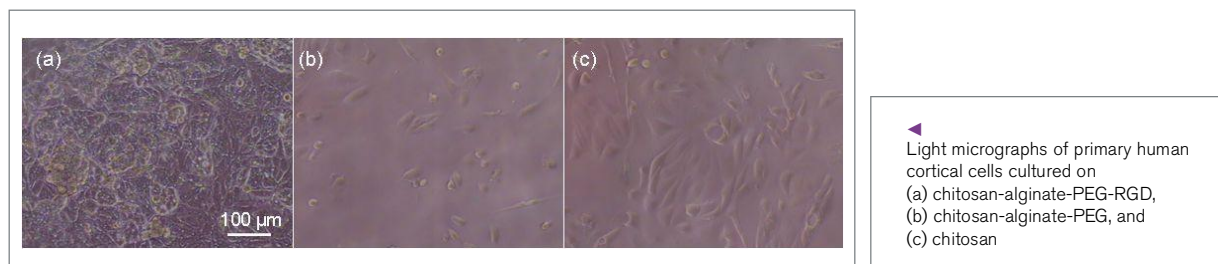
### Applications:

- Tissue engineering, 3D cell culture, 3D culture systems for high-throughput drug screening, stem cell expansion and differentiation.

# Cell and Tissue Engineering

## Functionalized Polyelectrolyte Complex Membrane and Coatings

Biomaterials for use in medical devices and tissue engineering need to be based on designed biological functionalities in order to deliver their unique functions. However, past generations of biomaterials are indiscriminate in ligand selectivity and attachment orientation. This is due to non-specific charge interactions between cells and surfaces that are often enriched by the absorption of proteins from the culture media. IBN has developed a three-tier membrane system that has a non-fouling surface upon which biological function, as exemplified by adhesion, can be presented at will. These materials consist of an underlying chitosan layer, an intermediate polyanion-polyethylene glycol (PEG) layer and a top layer that could be functionalized with biological ligands. Such a three-tiered system is useful as membranes for engineering epithelial organs such as kidney and liver. It is also suitable as a coating for both two- and three-dimensional surfaces, e.g. in porous structures that act as scaffolds for tissue engineering.



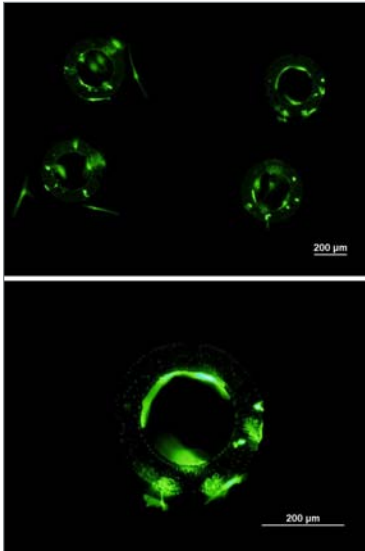
### Advantages:

- The cross-linking of the chitosan bottom layer (Tier 1) made use of silica condensation, which improves its adhesion to the hydroxyl-rich surface of substrates, such as the glass coverslips treated with "piranha" solution. In addition, silica condensation leaves the amino groups free for polyelectrolyte complexation, which is needed for strong adhesion to the middle polyanion-PEG layer.
- The middle polyanion-PEG layer (Tier 2) is suitable for the incorporation of proteins and other growth factors.
- The presence of maleimidyl groups in the top layer (Tier 3) is specific towards thiol groups and not amino groups, thus it will deactivate protein ligands that is attached to it (unlike EDC and NHS used in other membrane designs). In addition, such maleimidyl groups are relatively stable in water.

### Applications:

- Functionalized membrane/coatings for tissue engineering, implantable devices, artificial organs, and drug delivery.

# Cell and Tissue Engineering



▲ Endothelial cells growing within microfabricated structures.

## Cell Micropatterning and Microstructures from Two-Photon Laser Scanning

It has been reported that cells perform better in a 3D environment than on conventional 2D cell culture plates. Biomimetic 3D microstructures can provide the architecture to control cell-cell interactions and to guide cells to grow under conditions similar to that *in vivo*. To engineer functional tissue masses requires complex and high-resolution 3D microstructures for precise cell patterning to guide cell proliferation and polarization. Our two-photon laser scanning (TPSL) system comes with stationary operation that provides 10-time finer resolution than the cultured cell size to provide true 3D architecture and guide cells growth. Our photosensitive resin shows good biocompatibility and supports cell adhesion. The higher resolution of laser microfabrication also provides a higher degree of cell manipulation and control in three dimensions, which is important to induce cells to perform the proper function. At IBN, we have developed a cell encapsulation cum TPLS microfabrication process to eliminate existing cell seeding problems. In addition, our work presents a simple and flexible way to construct 3D microstructures for 3D cell culture and tissue engineering. These structures are fabricated in a manner that enables entrapment of cells at high density and viability, which further allow for rapid cell adhesion. The scaffolds are able to provide mechanical support and direct cell spreading according to the shape and curvature of the constructs. The material is cell compatible, biodegradable and non-toxic.

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### Advantages:

- The method confers the important advantage of being able to build complex 3D constructs, while providing the optimal microenvironment for tissue engineering and cell culture studies. Cell encapsulation cum TPLSP microfabrication process eliminate the existing cell seeding problems.

### Applications:

- Cell patterning, cell-based assays, tissue engineering.

# Cell and Tissue Engineering

## Collagen-Apatite Nanofoams as Bone Implants for Load-Bearing Applications

IBN researchers have developed a nanocomposite scaffold of collagen fibers and apatite crystals for use as bone implants. This scaffold is porous and has tunable mechanical properties to provide sufficient strength to support patients' lifestyles. It has excellent structural and chemical match to natural bone, making it resorbable by the body. It is also more osteoinductive than commercial scaffolds. Preliminary *in vitro* studies with MC3T3 osteoblast cells indicate excellent cell attachment and proliferation on this scaffold. Ectopic *in vivo* implantation on mice shows evidence of bone, tissue and blood vessel formation in the scaffold material. In addition, *in vivo* implantation of this scaffold into critical-sized defects on rat femur resulted in successful healing and functioning of the defect area without an external supporting cast.



### Advantages:

- Compared with other bone scaffold materials currently available in the market, IBN's scaffold has better mechanical properties, and microstructural and chemical match to natural bone. It also appears more osteoinductive than existing scaffolds.
- Control of the material's pore size and porosity can be easily achieved by controlling relevant parameters of the freeze-drying process, such as the freezing rate and water content of the sample.
- As IBN is able to produce the raw materials inexpensively in large quantities in-house, it will be possible to sell this scaffold at a much lower price than existing scaffolds.

### Applications:

- This scaffold material can be applied commercially as an osteoinductive load-bearing hard tissue implant.
- It can also be used for other tissue engineering applications.

# Cell and Tissue Engineering



▲ This implantable anorganic scaffold displays the original chemical and physical properties of natural bone.

## Three-Dimensional Bone Remodeling of Nanobiomaterials

Bone remodeling is one of the most promising bone repair techniques to be applied in orthopedic surgery. Biodegradable scaffolds play a key role in the repair process. Three-dimensional scaffolds provide a function similar to the extracellular matrix (ECM) for supporting osteoblast proliferation and differentiation. They also determine the shape and size of the new bone. Optimization of scaffolds and establishment of a reliable and abundant source of cells are our initial targets. Specifically, anorganic porcine bone (APB) has been developed and shown to stimulate osteogenesis both *in vitro* and *in vivo*. The natural anorganic scaffolds in a three-dimensional bone cell culture system work as an excellent implant material, demonstrating their potential for tissue-engineered growth of human bone.

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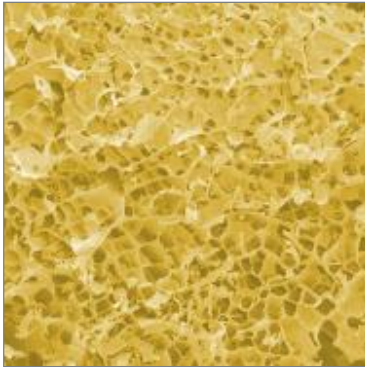
### Advantages:

- The bioprocessed anorganic porcine bone retains its natural three-dimensional scaffold. It is highly osteoconductive and osteoinductive.
- It has the original chemical and physical properties of natural bone, and is hence, biocompatible and can be implanted safely without the risk of viral infection or immunological response.
- Implants can be shaped easily for specific treatments.
- The bioprocessing technique is simple and low cost, making it highly efficient.
- The scaffold is made from the bone of animals that have been bred for consumption. This natural source is readily available.

### Applications:

- The technology is available immediately for use in *in vitro* cell culture for tissue engineering. The bioprocessed bone will provide a model to elucidate the natural mechanism of bone remodeling *in vitro*.
- The bone material has the potential to replace all existing bone scaffold materials as the scaffold maintains the original architecture and components that are most suitable for bone repair.

# Cell and Tissue Engineering



▲ Honeycomb structure of the hydrogel contains large numbers of water-holding cavities. The porous nature of the material also favors drug loading, and allows cells to grow within the pores.

## Peptide-Based Hydrogels for Drug Delivery, Tissue Engineering and Artificial Organ Applications

IBN's novel hydrogel-forming peptides (L- and D-form) are derived from natural amino acids. These ultrashort peptide amphiphiles (3-7 mers) self-assemble into complex fibrous structures when dissolved in water. While existing hydrogels are limited in their applications because of the high production costs involved, the simpler synthesis of IBN's peptides and their self-assembly into hydrogels make them more cost-effective, enabling them to be used for a wider range of medical and biotechnological applications. Target markets include the research community, hospitals, as well as medical and biotechnological companies.

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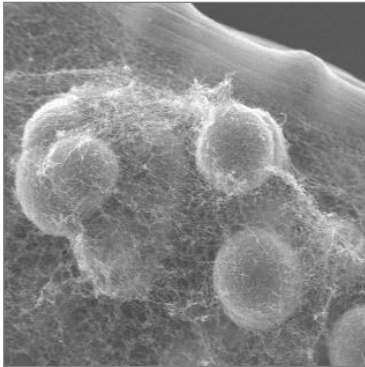
### Advantages:

- Retains up to 99.9% water content.
- Sufficiently high mechanical strength.
- Similar composition as extracellular matrix (ECM).
- Biocompatible towards a variety of human primary cells.
- Cost-effective production through self-assembly.
- Biodegradable material allows minimally invasive removal of the scaffold post-recovery.

### Applications:

- Biological scaffolds for cell culture and regenerative medicine.
- Patient-tailored tissue engineering for wound healing and grafting, plastic surgery and tissue replacement.
- Substitute for ECM.
- Biocompatible material for drug carriers or oral applications.
- Biological carriers in electronic devices such as biosensors.
- Cosmetics and packaging.

# Cell and Tissue Engineering



▲ Consistent microencapsulation for small scale application (< 1 ml).

## Device for Small-Scaled Encapsulation of Animal Cells

The encapsulation of animal cells is of great significance in biotechnology and medicine. For example, hepatocytes in microcapsules composed of ultrathin, semi-permeable membranes can have several applications, including their use in extracorporeal devices e.g. hybrid bioartificial liver-assistive devices. The encapsulation of cells in polymers such as alginate through alginate-gelation complex coacervation method has been developed previously, but preliminary results have been inconsistent. An alternative and more consistent two-step process has been devised, which involves calcium alginate droplets being incorporated into larger alginate gel spheres before reacting with a poly-L-Lysine to form a semi-permeable membrane. Sodium citrate was then used to liquefy the interior to form microcapsules. Unfortunately, sodium citrate has an adverse effect on hepatocytes and poly-L-Lysine and alginate are not very biocompatible. This invention overcomes such drawbacks by using collagen. It is a simple means for cell encapsulation with a semi-automated mechanism similar to the manual extrusion of immobilization mixture such as positively-charged collagen into hardening polyelectrolyte terpolymers of methylacrylamide, 2-hydroxyethyl methacrylate, and methyl methacrylate through complex coacervation.

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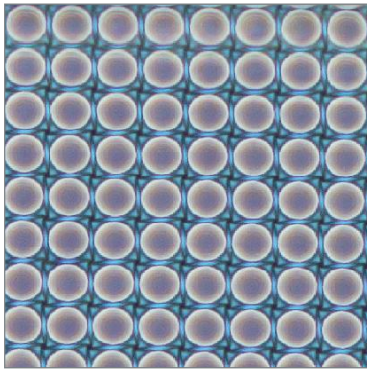
### Advantages:

- Current product in the market handles batch sizes with minimum volumes of 20 ml. This invention can handle volumes down to 1 ml, thereby reducing wastage of samples.
- Better maintenance of sterility.
- Encapsulation is much gentler to cells as no high voltage or organic solvents are used.

### Applications:

- Cell encapsulation for scientific and clinical research purposes.

# Cell and Tissue Engineering



▲ Hepatocytes micropatterned on microfabricated silicon nitride membrane.

## High-Throughput Cell-based Assays Fabricated with Integrated Silicon and Cell Culture Technologies

Cell-based devices have been used in many high-throughput pharmaceutical testing systems to study cell behavior, including cell growth, cell migration and cellular response to agents such as toxins, pathogens, drugs or other cells. The use of materials such as membranes within the cell culture environment has furthered the development of cell-based devices that may be useful in various applications. Many systems employ the use of polymer membranes wherein cells may adhere to the surface of the membrane or within the pores of the membrane, and their behavior may be studied. It is difficult to control and monitor the adherence of cells to many conventional polymeric membranes. While cells may adhere to the pores, they may also adhere to locations in between pores, which prevent the growth of ordered tissue. This may also be undesirable for certain applications. In this invention, the immobilization of cells with respect to the membrane is controlled such that adherence is achieved only in regions within individual pores, rather than in areas between pores.

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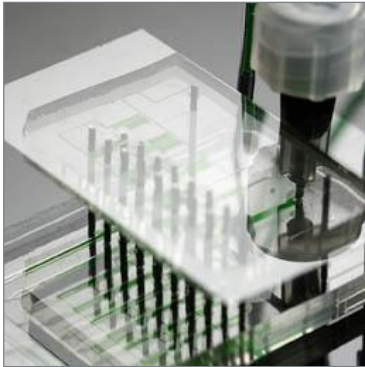
### Advantages:

- Greater control of the pore distribution, pore size and cell adherence.
- Comparable to cell suspension systems (e.g. 1536 well plates).
- It is easier to integrate into a cell-based, high-density, perfusion cell culture system.
- It maintains cell viability for a longer time.
- It is based on silicon microfabrication techniques, and is thus compatible with standard industrial manufacturing processes.
- Transmission of visible light through the cell/collagen/silicon nitride membrane is more than 50%.

### Applications:

- Cell-based arrays, microfluidic systems, tissue engineering, artificial organs and implants, and extracorporeal medical devices.

# Cell and Tissue Engineering



▲ Microfluidic channels with micropillar array for three-dimensional mammalian cell culture.

## Engineering of Microliver Tissues

Complex liver tissue can be engineered *in vitro* by controlling various aspects of the liver cell microenvironment, such as cell-cell and cell matrix interactions, 3D topography, and mechanical signals. This is achieved through the combinatorial use of novel micro-technologies, 3D hydrogels, surface modification techniques as well as controlled delivery of soluble factors to precisely manipulate chemical and mechanical cues that modulate cellular responses. Biomaterials and novel microfabrication/microfluidic technologies are integrated to generate precisely controlled cell-containing structures that preserve the functions of liver cells as prototype microliver tissues.

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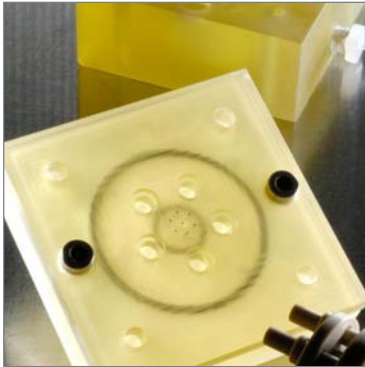
### Advantages:

- Non-toxic to cells through the use of a mild and aqueous technique.
- Minimized exposure of live cells to harsh conditions during fabrication as live cells are immobilized instantaneously by an *in situ* extracellular matrix (ECM) gelation process.
- Ideal for system integration for *in vitro* testing model as it can be easily incorporated into current microfluidic systems.
- Ideal for hepatocyte-based screening/testing due to the stabilizing effect of a hybrid bioactive substratum that allows the cell spreading phase of up to a week.
- Versatility in designing liver tissue construct due to:
  - General method for creating 3D cell constructs applicable to all cell types (single or combination of cell types).
  - Immobilization of multiple cell types simultaneously via multiple laminar streams.
  - Decoupling of 3D cell immobilization and 3D cell-matrix support.
  - Configurable physical and chemical properties of the ECM to allow the extracellular microenvironment to be engineered for optimal cell functions.
- High level of biomimicry achieved by exploiting the existing inherent ECM and microvasculature of the liver.

### Applications:

- Cell biochips containing arrays of live cells that are useful in high-throughput screening (HTS), toxicology, biosensors and cancer diagnostics.
- *In vitro* models of physiological tissues, e.g. for drug testing, cancer metastasis studies.
- Scaled up for bio-artificial liver-assisted devices to help patients with acute liver failure, e.g. implantable tissues and in extracorporeal devices.

# Cell and Tissue Engineering



▲  
Intra-tissue perfusion bioreactor for thick liver slice culture.

## Intra-Tissue Perfusion Using Microneedle Array for Engineering Thick Tissue Constructs

Long-term culture of thick tissue constructs for regenerative and tissue engineering applications is limited as a result of mass transport to the deep layers of tissue constructs. To overcome this problem, IBN has developed an intra-tissue perfusion system to deliver nutrients directly into the core of thick tissues using microneedles as a delivery conduit. This system has potential to re-build large tissue-engineered constructs for organ replacement *ex vivo* and pre-form vascular beds for more efficient interface with the host. It is also an ideal tool for *in vitro* biological investigations and as a drug testing platform with the advantage of controllable hemodynamic and cellular microenvironments.

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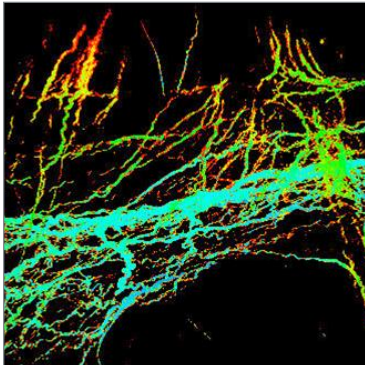
### Advantages:

- Excludes the necessity of hepatocytes isolation and stimulation of cells to maintain high functionality with a variety of growth factors, scaffold design and co-culture.
- Microneedles perfusion enhances the uniform distribution of perfusion media, which subsequently ameliorates the viability and functionality of the tissue over a long-term culture.
- Microfabrication techniques enable design and development of a range of microneedles of varying size, array distance and shape, which permit versatility in experimental designs.
- Utilization of microneedles can potentially facilitate the introduction of different drugs at different regions of the liver, and investigate the interaction of the cells from different regions with respect to the drugs introduced.

### Applications:

- 3D tissue biochip and platform for absorption, distribution, metabolism and excretion/tox investigations, high-throughput screening (HTS), bioimaging and *in vitro* model for biological investigations and cancer diagnostics.
- Potential to be used for nutrient delivery to improve mass transfer to engineer large tissue constructs.

# Cell and Tissue Engineering



▲ Imaging extracellular matrix in three dimensions.

## Sensitive Second Harmonic Generation Microscopy for Quantifying Matrix-Related Tissue Dynamics and Diseases

The classical methods for studying the anatomy of cells and tissues utilize the histological staining and microscopy imaging. Such methods are ideal for observing information such as size and shape of certain organelles, cells, and tissues. However, the technique is restricted to very thin slices of tissue due to effects of dye diffusion and optical penetration for imaging. This limitation poses a problem in tissue culture work, where numerous layers of cells are needed to keep structural and functional integrity. In addition, the effect of staining on cell behavior has always been a concern as it introduces more complication to cell physiology. Recently, non-linear optical microscopy has been developed to address these drawbacks, but this technique is plagued with fundamental problems such as light scattering and weak signals. A lot of important information such as the extracellular matrix (ECM) has always been overlooked with classical methods due to the lack of sensitivity and spatial resolution. ECM dynamics is closely related to many pathological conditions including chronic inflammation, fibrosis and tumor cell invasion. Recently, IBN researchers were able to overcome this barrier and optimized the optical settings to achieve even higher sensitivity. This technical breakthrough opened the door to a new generation imaging system for applications such as standardized clinical diagnosis on liver fibrosis, studies on fine ECM remodeling and its physiological implication, and non-invasive imaging tool for cancerous cell identification.

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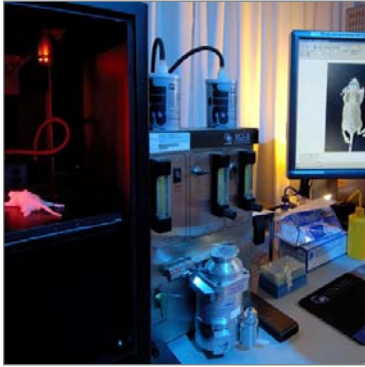
### Advantages:

- Ability to characterize collagen in 3D thick slices (more than 50  $\mu\text{m}$ ).
- No need for staining.
- Whole liver lobe or organ can be imaged in backscattering configuration, no need for tissue slicing.
- Better spatial resolution and higher sensitivity than histological staining.
- Imaging is performed under physiological conditions.
- The imaging process is completely standardized, reproducible.

### Applications:

- Diagnostic tool for quantitative analysis of fibrosis and collagen-related liver diseases.
- Research tool to monitor tissue structure dynamics during liver tissue-based drug screening.
- Clinical tool to guide the excision of tumors.

# Cell and Tissue Engineering



▲  
*In vivo* biological imaging of transgenic mouse model.

## ***In Vivo* Biological Imaging**

Non-invasive detection of gene expression in live animals is now possible with new imaging hardware and imaging tags. Various gene reporters are being explored for imaging purposes, in order to develop novel platform technologies for non-invasive *in vivo* imaging of living animals in a tissue-specific manner (with a special focus on the nervous system), through the use of a gene promoter in transgenic mouse models. The non-invasive imaging platform (transgenic mouse models plus imaging hardware) can be used to screen compound toxicity and drug efficacy in real-time.

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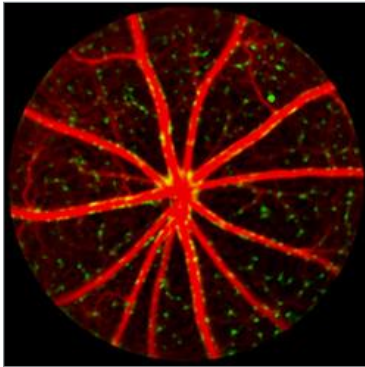
### **Advantages:**

- This technology is non-invasive and real-time in nature.
- It produces high-quality data, and is cost-effective, as compared to the conventional animal experimental protocols.

### **Applications:**

- This invention can be used for the toxicity screening of drug and chemical compounds, pesticides, insecticides, and food additives; the screening of drug candidates; as well as the animal modeling of Parkinsonism, especially in the developmental and environmental aspects of the Parkinsonism.

# Cell and Tissue Engineering



▲ Interaction between retinal glial cells and vasculature in a mouse model.

## Imaging Retinal Disorder in Early Diabetes

IBN's non-invasive retinal imaging technique offers a distinctive advantage for longitudinal examination of the retina to assess the health and disease status of the eye and brain in animals and humans. This technical innovation opens up possibilities for the diagnosis of eye disorders in human subjects, as well as experimental therapeutic development in animal models of human eye diseases. Target markets include hospitals and eye clinics, biotechnology, pharmaceutical and chemical industries, contract research organizations and regulatory bodies.

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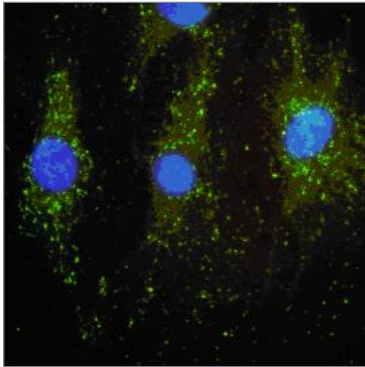
### Advantages:

- Non-invasive retinal imaging is possible with or without imaging probe.
- Early detection and diagnosis of retinopathies in human eyes enables prevention and early intervention.

### Applications:

- Early detection and diagnosis of retinopathies in human eyes.
- Diagnosis of eye disorders in human subjects.
- Experimental therapeutic development in animal models of human eye diseases.
- R&D of ocular drugs.

# Cell and Tissue Engineering



▲  
IBN's nanoparticles yielded much higher GFP and luciferase expressions in HEK293 cells as compared to PEI.

## Hepatic Stellate Cells Express Cathepsin S and Other Key Molecules Required for Antigen Presentation

The liver is a complex organ and is used for the detoxification of waste products in the body. Many antigens pass through the liver via the portal vein. The liver has to respond either with tolerance to avoid autoimmunity or with immunity to ward off infectious agents. Recent studies have shown that hepatic stellate cells (HSCs) play an important role as mediators of hepatic fibrosis by producing profibrotic cytokines and extracellular matrix (ECM) proteins. HSCs also play a role in antigen presentation. It has been shown by IBN researchers that Cathepsin S is required for antigen presentation in HSCs, and thus, this serves as an ideal drug target for the treatment of certain liver diseases such as autoimmune diseases, organ rejection, fibrotic liver diseases and carcinoma.

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### Advantages:

- First documented evidence of Cathepsin S involvement in both the immuno-modulation (stimulatory or inhibitory) and the modulation of ECM in the liver.

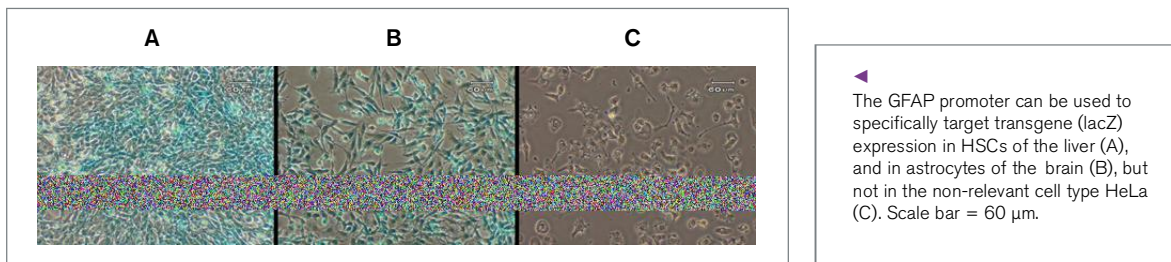
### Applications:

- Drug targeting for the therapeutic intervention of
  - Hepatic immuno-tolerance to foreign materials, self-antigens, autoimmune disorders, liver transplantation and food allergy.
  - Hepatic modulation on ECM remodeling in the liver, cancer metastasis and tumor angiogenesis.

# Cell and Tissue Engineering

## Targeting Hepatic Stellate Cells for Anti-Fibrosis Gene and/or Drug Therapy

Hepatic stellate cells (HSCs) are activated during fibrosis and have been found to have elevated levels of certain genes. This technology relates to a promoter system that can be used to direct HSC-specific gene expression. The promoter expression system can be used to study inhibition of HSC activation and fibrosis, develop anti-fibrotic therapy, and deliver gene/drug specifically to HSCs. For example, the promoter could be linked to a reporter gene to genetically engineer an *in vitro* HSC-based system for studying hepatic fibrosis, or it could be linked to a therapeutic cDNA to develop an anti-fibrotic gene therapy system. Alternatively, the promoter could be used in a siRNA-based therapeutic vector to develop an anti-fibrotic siRNA therapy system. It may also be linked to a high-affinity ligand for drug delivery.



### Advantages:

- The 2.2kb human glial fibrillary acidic protein (hGFAP) promoter is capable of directing HSC-specific expression.
- The promoter can be linked to different types of genes to genetically engineer different types of *in vitro* HSC-based systems for various applications.

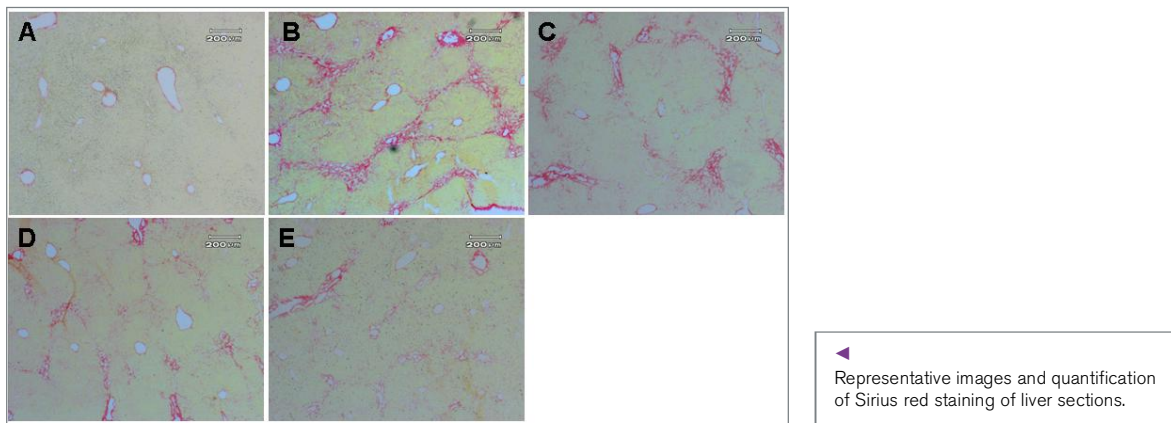
### Applications:

- Study of hepatic fibrosis, development of anti-fibrotic therapy, high-throughput screening for anti-fibrotic drug compounds, and targeted delivery of genes or drugs specifically to HSCs.

# Cell and Tissue Engineering

## Imidazolium Salt Compounds as Therapeutic Agents

Imidazolium salts (IMs) are aqueous compounds known for their anti-oxidative, anti-inflammatory, anti-fibrotic, anti-cancer and neuroprotective properties. These small molecules (< 500 daltons) may easily pass through the blood-brain-barrier and blood-retina-barrier, and exert neuroprotective properties *in vitro* and *in vivo*. IBN's recent discovery of the biomedical applications of IMs has opened up potential new opportunities to combat various degenerative diseases.



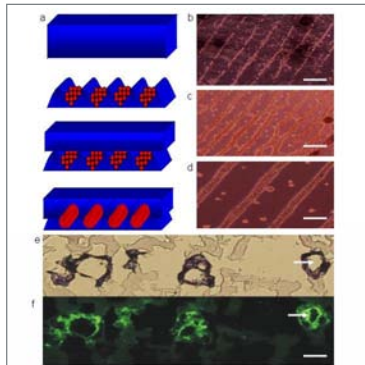
### Advantages:

- Unique IP position for the biomedical applications of IMs.
- Biomimetic compounds with relatively straightforward chemistry.
- Able to pass through the blood-brain barrier and blood-retina barrier easily.

### Applications:

- Central nervous system disorders (Parkinson's disease, Alzheimer's disease, multiple sclerosis, etc.).
- Eye disorders (diabetic retinopathy, age-related macular degeneration, etc.).
- Peripheral neuropathies.
- Fibrosis (liver, lung, kidney, pancreatic, etc.).
- Cancer (liver, lung, kidney, pancreatic, brain, etc.).

# Cell and Tissue Engineering



▲  
Controlled formation of tubules through hydrogel molding.

## Controlled Formation of Biological Tubule System

Various epithelial organs such as the lung and kidney are made up of networks of epithelial and endothelial tubules. In order to engineer such organs, it would be desirable to control the formation of tubules with regard to their shape, spatial orientation and interconnectedness. Tissue engineering approaches aim to engineer cell or tissue constructs via a combination of cells and biomaterials *in vitro*, which are subsequently implanted to restore lost tissue in the body. In addition, tissue engineering approaches can be applied to develop *in vitro* systems that allow the investigation of biological processes, such as the self-assembly of cells, in order to gain insights into the processes of tissue formation during embryonic development. However, the development of tubular structures has been mostly achieved by seeding cells or cell aggregates into scaffold structures or hydrogel sponges with limited control of tubule formation. At IBN, we have developed a novel system for the controlled formation of tubule system through the use of kidney cells, extracellular matrix and collagen gel materials.

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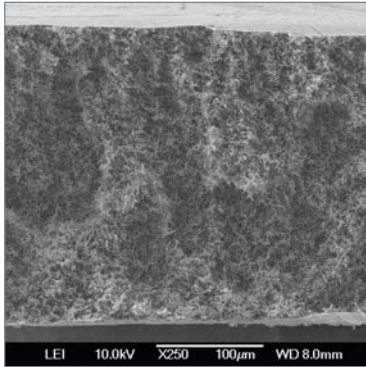
### Advantages:

- Ability to generate epithelial and endothelial tubules of significant length (> 1cm) and regular anatomical shape.
- More biomimetic than structures produced by current techniques.
- More control in the formation and orientation of tubule systems.

### Applications:

- Engineering of implantable tubule-containing tissues, such as kidney and vessel systems.
- Engineering of prevascularized microtissues and macrotissues for epithelial tissue engineering.
- Engineering of tubular systems and organ precursor structures derived from adult and embryonic stem cells.

# Cell and Tissue Engineering



▲  
IBN's patented PS-FC membrane.

## Porous Membrane for Biohybrid Organs

Organ failure patients can be treated using artificial medical devices but these treatments often bring about harmful side effects. For example, hemodialysis can replace the filtration function of the kidney, but patients often suffer from amyloidosis, in which protein is deposited in specific organs or throughout the body after only a few years of dialysis. As a result, organ replacement is still the best long-term solution for these patients. However, there is a lack of organs available for transplant globally and there are immuno-compatibility issues in some cases. Biohybrid organs (BHOs), which combine known mechanical functionalities (e.g. filtration) and primary organ cells (e.g. renal tubule cells), is thus the next best alternative for organ replacement. Currently, the bottleneck in developing BHOs with high biospecificity and biocompatibility lies in the inability for primary organ cells to retain their functions. Part of the reason for this is that these cells are usually immobilized onto membranes that are not developed for their specific functions. IBN researchers have addressed this drawback by developing a novel porous polysulfone-Fullcure™ 700 (PS-FC) membrane system. This membrane has open and interconnected micron-sized pores, making it highly permeable with attractive biofunctional characteristics e.g. sharp molecular weight cut-off (MWCO). It also possesses high filtration and diffusion fluxes as well as good mechanical strength, biocompatibility and formability. In addition, it appears to improve the development of monolayer kidney cells. These characteristics make IBN's technology very useful in the development of a bioartificial kidney.

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### Advantages:

- Can be tailor-made to suit different specifications, e.g. sharp molecular weight cut-offs, steam-sterilized, and surface functionalized.
- Versatile for BHO printing as it can be fabricated in a wide range of shapes, sizes and permeabilities.
- Simple and inexpensive to produce, and is easily scaled-up for mass production.
- Can be incorporated in PolyJET polymer printing process.

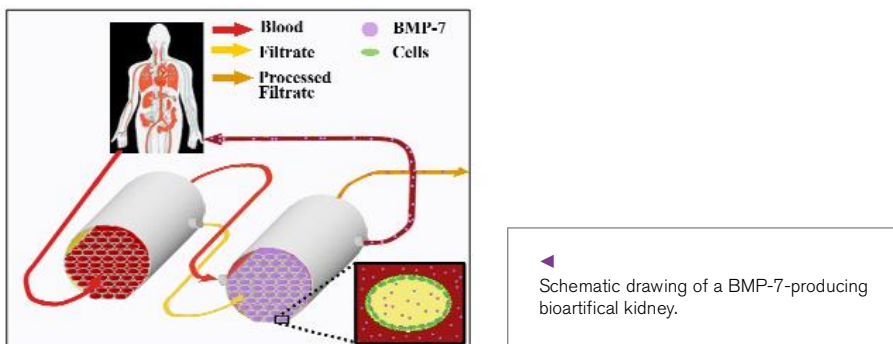
### Applications:

- Hemodialysis.
- Water treatment or purification system.
- 3D tissue engineering scaffolds.
- Artificial BHO printing.

# Cell and Tissue Engineering

## Construction of BMP-7-Producing and BMP-7-Releasing Bioartificial Kidneys

The growth factor bone morphogenetic protein (BMP)-7 has been shown to be effective in the treatment of acute and chronic kidney disease in animal models. BMP-7 also has positive effects on conditions often associated with chronic kidney disease, such as renal osteodystrophy or vascular calcification. FDA approval has been obtained for the use of BMP-7 to treat bone disease, and there are commercially available products that release BMP-7 locally at the site of bone defects. To treat kidney disease and associated conditions, systemic delivery of BMP-7 is required. A major obstacle in the development of BMP-7-based clinical applications and products for treatment of kidney disease is the short serum half-life of BMP-7 (~ 30 minutes). One way to address this problem would be the development of a device that continuously releases BMP-7 into the patient's bloodstream. IBN is developing bioartificial kidneys that contain a hemofiltration unit in series with a bioreactor unit seeded with human renal cells. The renal cells perform transport functions, and release bioactive factors into the patient's bloodstream. Bioartificial kidneys with a comparable design have already been applied in Phase I/II and Phase II clinical trials that enrolled critically ill patients with acute renal failure. Significant improvement of long-term survival was observed. IBN's device would be suitable for prolonged or continuous treatment of patients suffering from acute renal failure. Normally, the renal cells applied in bioartificial kidneys do not secrete BMP-7. Genetic modification of the cells within the device allows for continuous delivery of BMP-7 to the patients during treatment. Continuous BMP-7-delivery can also be achieved by controlled release of this factor from other components of the device. Apart from the expected beneficial effects on the patients, BMP-7 will also improve cell performance within the device, which is critical in bioartificial kidneys.



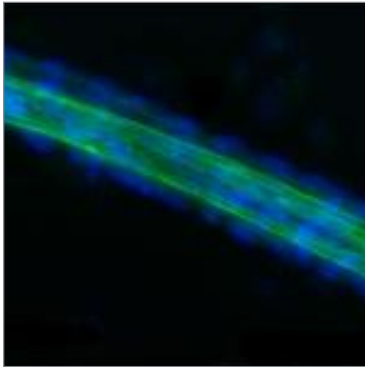
### Advantages:

- The major obstacle associated with systemic delivery of BMP-7 to kidney patients (short serum half-life) has been overcome.
- The additional costs for BMP-7 treatment will be low.

### Applications:

- Treatment of acute renal failure.
- Potential for treatment of chronic renal failure and associated conditions.

# Cell and Tissue Engineering



▲ Human renal tubule generated *in vitro* on a 2D surface in a gel-free system.

## Human Kidney Tubules Generated *In Vitro* on 2D Surfaces in a Gel-Free System

Renal tubules are essential for kidney function, as they reabsorb most of the water from the glomerular filtrate (about 180 l/day) and transport organic compounds and drugs, which cannot be efficiently cleared by ultrafiltration, into the glomerular filtrate. These roles make them the primary targets of nephrotoxic agents and drugs. Hence, cells derived from the renal tubule are widely used for *in vitro* nephrotoxicology. Monolayers of renal tubule-derived cells are also applied in basic research to study cellular transport processes and renal physiology. However, renal tubule functions and processes that compromise tubular functions and integrity can only be studied to a limited degree with monolayer cultures. The procedures for isolating kidney tubules from renal tissues are labor-intensive, and isolated tubules have only a limited lifetime of about 2 hours. Three-dimensional gel-based systems for generating kidney tubules *in vitro* have been developed, but the tubules are formed at variable positions within the gel and always remain embedded within it. The surrounding gel compromises high-resolution imaging, and the tubules are not directly accessible for manipulation. Drugs or nanoparticles (for nanotoxicology studies) cannot be applied to them in a controlled manner. To overcome these limitations, IBN developed a method to generate human kidney tubules from primary human renal tubule cells *in vitro* on two-dimensional surfaces in a gel-free system.

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### Advantages:

- Human renal tubules generated in a gel-free system are exposed on the substrate surface, and are thus directly accessible for manipulation and controlled application of drugs, nanoparticles or other compounds.
- Unfixed specimens, with a lifetime of at least one day, can be easily imaged by high-resolution light microscopy in order to study tubular functions and transport processes.
- High-resolution light microscopy is not compromised by any surrounding gel.
- Results are not compromised by interspecies variability or altered functionality of immortalized cells.

### Applications:

- *In vitro* nephrotoxicology and nanotoxicology, drug screening, tissue engineering, renal physiology.
- The system can be used to study the regeneration of renal structures and fibrotic processes after organ disruption, and the influence of drugs on such processes.

## Notes

## Notes

## Notes

