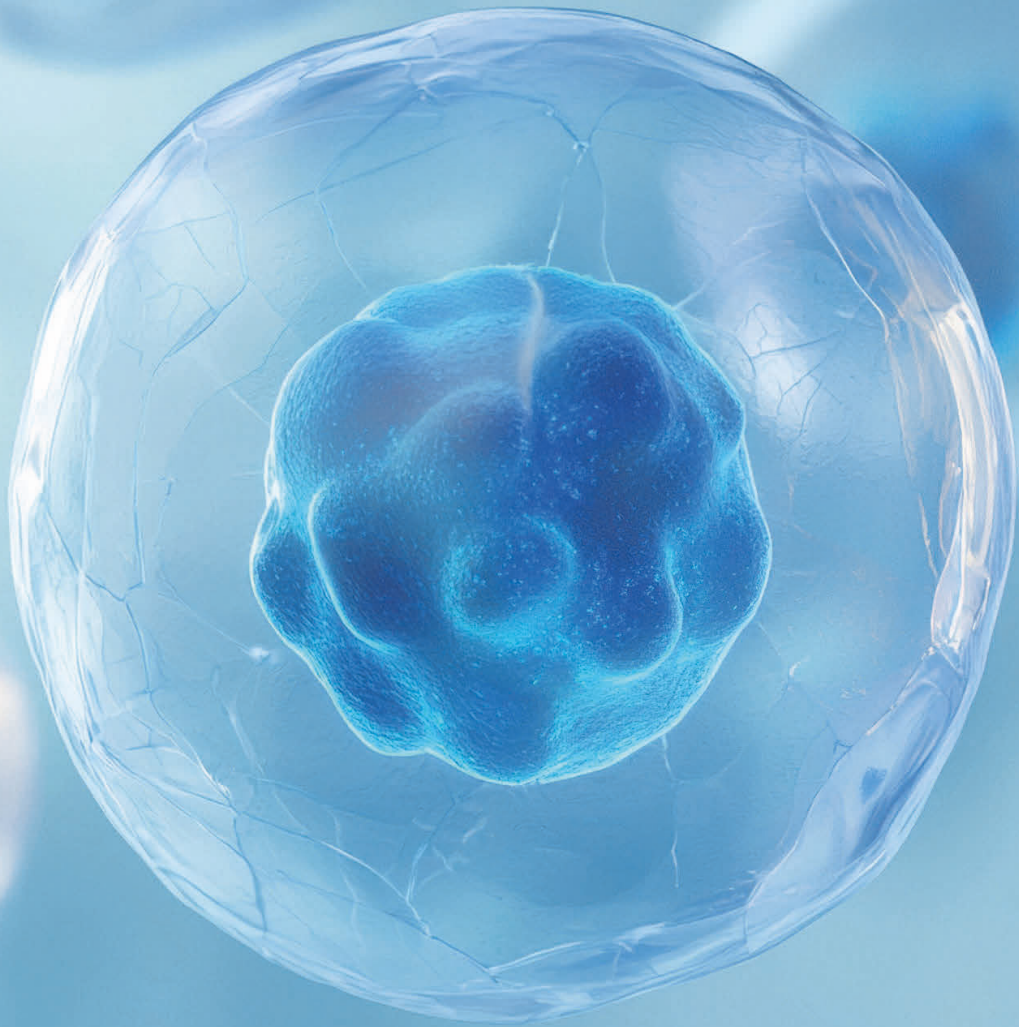




Re:ancel Bio-CNF

Plant-based New Biomaterial for Advanced 3D Cell Culture

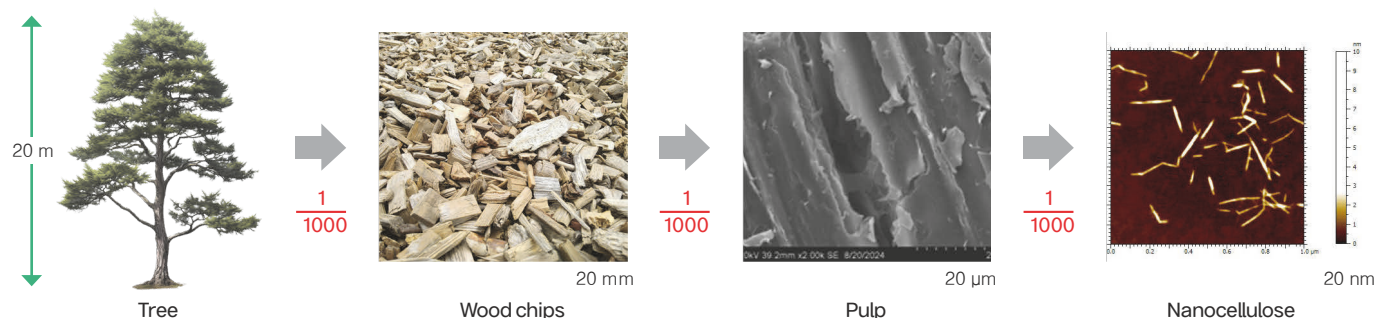


Re:ancel Bio-CNF



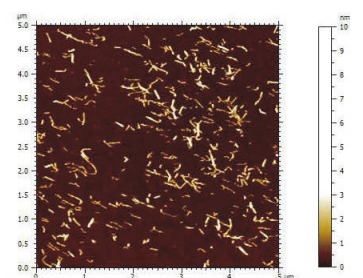
ANPOLY is a high-tech materials platform company that develops and commercializes eco-friendly, high-performance materials derived from plant-based nanocellulose. Re:ancel Bio-CNF is a next-generation extracellular matrix that ensures high uniformity and reproducibility while reducing immune responses, providing a stable cell culture environment. Engineered for three-dimensional cell culture and organoid research, it supports reliable drug testing and regenerative medicine applications, establishing a new standard in molecular cell biology as a sustainable bio-product.

CNF?

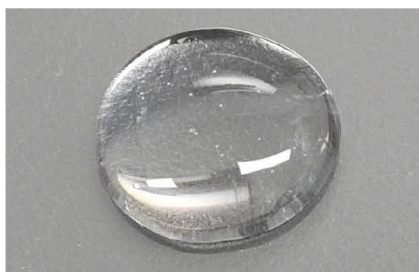


CNF (Cellulose NanoFiber) is a new eco-friendly fiber made by breaking down cellulose from trees or plants into nanoscale fibers. CNF is extremely strong while weighing only one-fifth as much as steel and offers unique advantages such as transparency, biodegradability, and high thermal stability, making it a promising next-generation material. ANPOLY has proprietary technology to produce nanocellulose from wood pulp, rice husks, and coffee grounds, and has developed and commercialized secondary battery binders, separators, biocomposites, and ECMs using this nanocellulose.

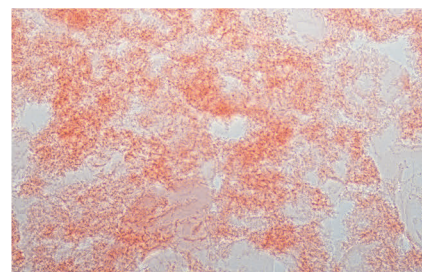
Re:ancel Bio-CNF



Bio-CNF



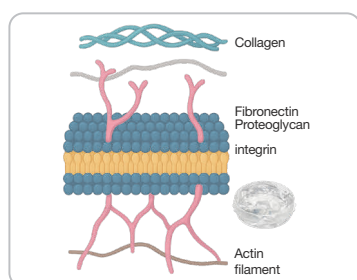
Appearance



Congo red staining

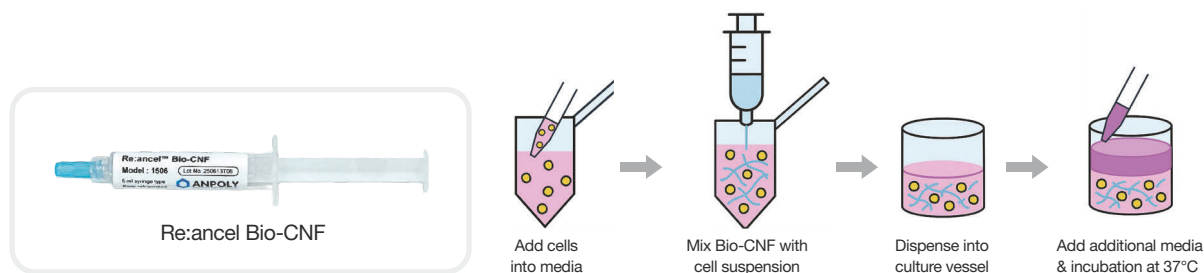
- ✓ Form an ECM with high uniformity to provide reproducible cell culture results
- ✓ Customized research with surface modification and protein binding, with the flexibility to expand multiple features

Similarity to Traditional ECM



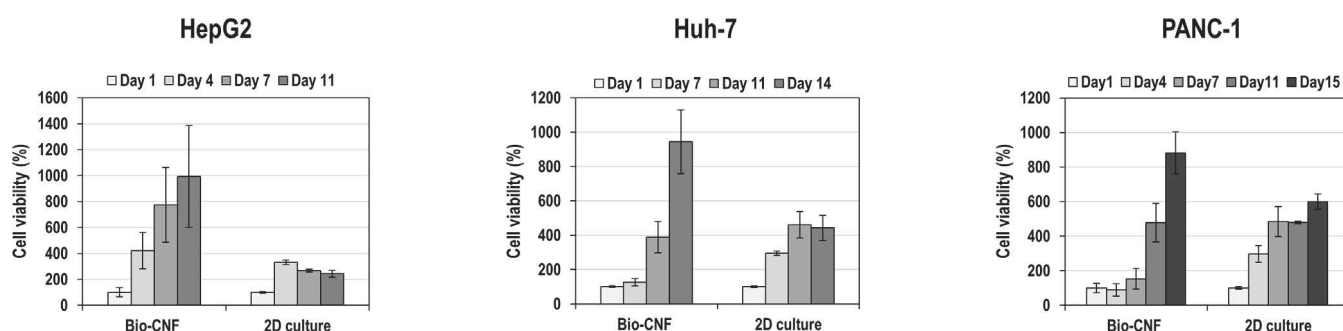
Re:ancel Bio-CNF is a non-animal-derived alternative to conventional ECM materials, reproducing key ECM properties such as high-water content and three-dimensional fibrous structures that promote stable cell proliferation and organization. Unlike animal-derived products, Re:ancel Bio-CNF shows no batch-to-batch variation and delivers 15~20% greater cell growth potential in culture compared to conventional products. Drug efficacy and toxicity tests based on this material have also demonstrated outstanding performance.

How to Use



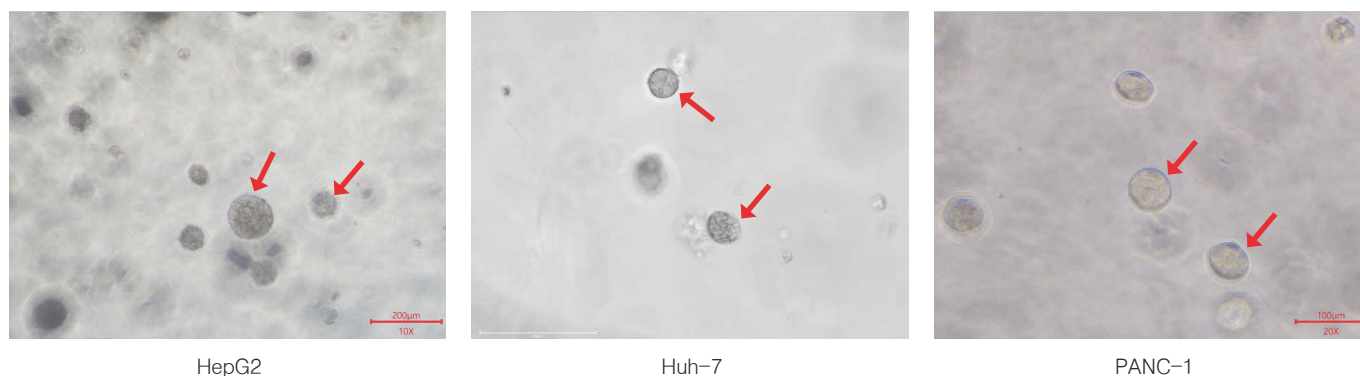
- ✓ The 1% Bio-CNF is diluted in the cell culture medium to 0.3~0.5%, depending on the cell type
- ✓ Count the desired number of cells, then mix them with Bio-CNF and seed them onto a plate
- ✓ After dispensing Bio-CNF mixed with cells into the plates, stabilize the samples in a 37 °C incubator for 20~30 min
- ✓ After stabilization, the cells were seeded into the prepared plates, and cell culture medium was added before incubation

Cell Viability



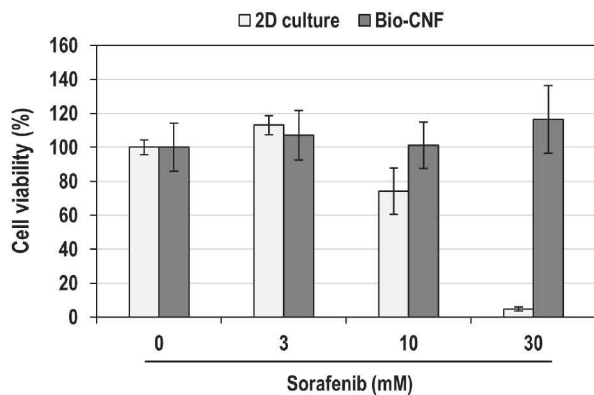
- ✓ After 3D culture of HepG2, Huh-7, and PANC-1 cell lines in 0.5% Bio-CNF, cell viability was comparatively evaluated against 2D culture over the culture period
- ✓ Cell viability was measured using the CellTiter-Glo 3D Cell Viability Assay
- ✓ Higher cell viability in 3D cell culture based on Bio-CNF was evaluated compared to 2D cell culture

Cell Morphology Image



- ✓ Cell morphology images of HepG2, Huh-7, and PANC-1 cell lines cultured in 0.5% Bio-CNF were captured
- ✓ The cells were observed on day 7 of incubation using an inverted microscope (Zeiss Axio Vert A1)
- ✓ The round-shaped spheroid forms a three-dimensional spherical aggregate

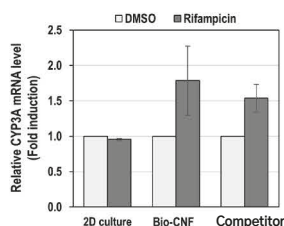
Attenuated Sensitivity to Sorafenib in 3D Hepatocyte Culture



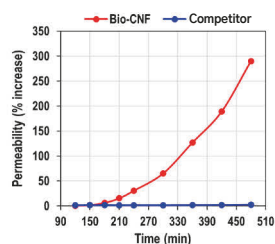
- Drug responses were evaluated in 2D HepG2 cultures and in Bio-CNF-based 3D models
- Cell viability was measured on day 5 of culture following treatment with varying concentrations of Sorafenib
- In 3D Bio-CNF cultures, resistance patterns resembled those observed in vivo, likely due to limited drug infiltration

Comparison with Competing Products

Expression of CYP3A4



Transparency of FITC-Dextran



- Comparison with competing products (animal derived ECM)

- Expression of CYP3A4

HepG2 cells were treated with Rifampicin to induce CYP3A4 expression. Real-time PCR analysis was conducted to assess expression and evaluate equivalence with competitor products.

- Transparency of FITC-Dextran

In the Transwell system, FITC-Dextran was treated in the upper chamber. Permeability to the lower chamber was measured over time to evaluate performance relative to other products

- Autofluorescence

Bio-CNF shows no intrinsic autofluorescence, with emission levels at the commonly used excitation wavelengths of 405 nm and 488 nm comparable with those of distilled water, the negative control. In contrast, competitor's product exhibited fluorescence patterns similar to the positive controls, Hoechst 33342 and FITC-dextran. This low background signal of Bio-CNF enables clearer fluorescence imaging and more reliable analysis, thereby making it an ideal scaffold for advanced 3D cell culture and precise drug evaluation.

Autofluorescence

