# Gelacell™ - PLLA 10x10 scaffold

*Bringing cell culture to the next dimension*

## Product Description

Gelacell™ is a unique, 3D microfibrous scaffold specifically engineered for advanced in vitro 3D cell culture and tissue engineering applications. Designed as a non-woven, highly porous scaffold, Gelacell™ offers exceptional biocompatibility and non-toxicity across a variety of cell types. This product includes a 10 cm × 10 cm scaffold sheet that offers a 3D structure allowing cells to grow in a more physiologically relevant manner, promoting cell-cell interactions, tissue-like organization, and cell polarization, which are challenging to achieve in traditional 2D cell culture. The sheet can be used in its presented format or can be cut into desired shape and sizes depending on the user's experimental needs and setups. The scaffold is perfectly tailored for the cultivation of complex systems like cell microenvironments, tissue regeneration, and organoids, Gelacell™ also makes a robust platform for drug screening and other cell culture investigations. The optimized design of the scaffold ensures thermal, chemical, and mechanical stability, while also providing substantial swelling capabilities and porosity, allowing efficient nutrient diffusion, and thereby preventing cellular waste build up.


Figure 1. Gelacell™ scaffold in 10 cm x 10 cm size.

## Product Features

- 3D architecture with a substantial available surface area.
- Minimal modifications required for 2D to 3D culture transition.
- Stable mechanical properties.
- 10 cm × 10 cm scaffold can be cut into desired shapes and sizes.
- Flexibility of the scaffolds facilitates easy handleability.
- Porous structure promotes cell migration and efficient diffusion of nutrients, solutes, and gases.



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- Compatibility with a variety of cell lines and culture conditions.
- Biocompatible polymers, diverse and widely accepted in cellular applications.
- Sterilized by Gamma irradiation and will remain sterile until the pack is opened.
- Storage at room temperature.

## Product Datasheet

| Product information | Gelacell™ |
|---|---|
| Product code | GC0805RN-SC-C |
| Polymer | Poly-L-lactide (PLLA)<br>(Pharmaceutical grade) |
| Appearance (dry) | White |
| Appearance (swelled in water/PBS) | White<br>(Translucent) |
| Fiber orientation[1] | Random |
| Mean fiber diameter[1] | 0.4 – 1.4 µm |
| Thickness | 140 - 230 µm |
| Area density | 3.0 – 9.0 g/m$^2$ |
| Porosity[2] | 95% - 99% |
| Wettability[3] | Hydrophilic<br>The material uptakes water, and compounds that dissolve in water due to the porosity and capillary forces wherever the material is soaked in a solution and incubated. |
| Absorptivity in PBS (pH 7.4 at 37°C)[4] | 600% - 1200%<br>The material was kept soaked for 24 hours in PBS at 37°C |
| Degradation (in PBS at 37°C)[4] | Up to 20% |
| pH (of PBS solution after 24 hours)[4] | 7.3 – 7.5<br>without observable changes |
| %Elongation[5] | 20% - 45% |
| Elastic modulus[5] | 400 – 1300 kPa |
| Ultimate tensile strength[5] | 80 – 220 kPa |

1. Information is derived from SEM images.
2. Porosity is determined by comparing the apparent densities of scaffolds to the bulk densities of PLLA.
3. Wettability is determined by visual observation when water/PBS was added dropwise on top of the scaffolds after washing the scaffolds with 70% ethanol. Ethanol washing is highly recommended as PLLA is inherently hydrophobic in nature.
4. Degradation and absorptivity tested in PBS solution. Absorptivity evaluates solution uptake, while degradation is tracked as weight reduction over time with bi-daily PBS renewal.
5. Tensile strength measured using a specific in-house protocol, conducted in dry conditions.

## Morphology of PLLA scaffold

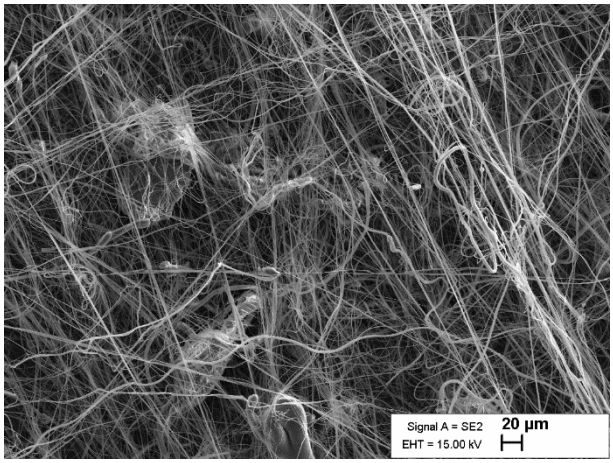


Figure 2. SEM image showing fiber morphology and porosity.

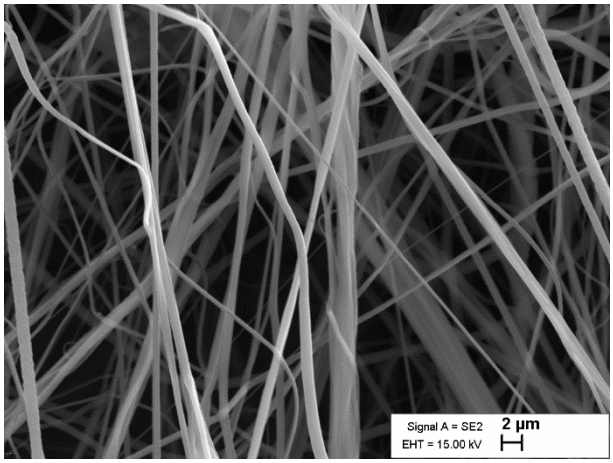


Figure 3. SEM image showing fiber diameter and distribution.

The morphology of fibrous scaffolds plays a pivotal role in its interaction with eukaryotic cells derived from humans and animals. The unique structure of microfibers shown in Figure 2 and Figure 3, mimicking the extracellular matrix, offers a high surface area, and interconnected porous network. This morphology provides an advantageous environment for cell adhesion, proliferation, and differentiation.

## Biocompatibility of PLLA Scaffold

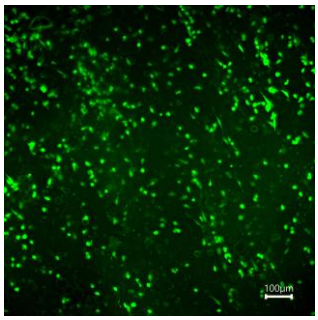

Figure 4. Hela cells after day 1.

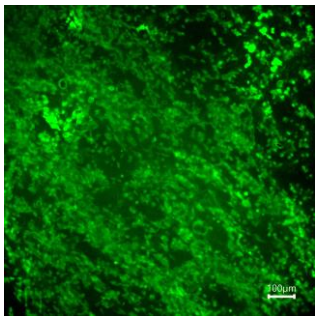

Figure 5. Hela cells after day 3.

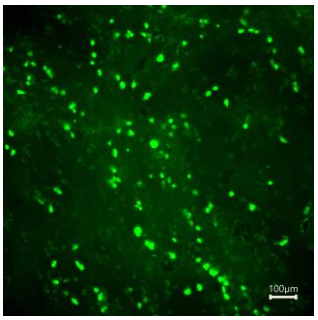

Figure 6. Huh7 cells after day 1.

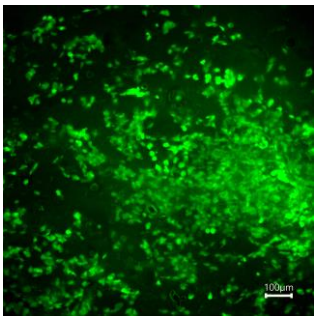

Figure 7. Huh7 cells after day 3.

The scaffolds exhibit promising biocompatibility with various cell types, including HELA, HEK293, HUH7, HEPG2, U2OS, BHK21, and C2C12. Their biomimetic design and specialized polymer composition enhance cell-material interactions, supporting key processes like cellular attachment and spreading - crucial for cell growth and function. HELA and HUH7 cell lines show adherence, proliferation, and infiltration within the scaffold structure (as depicted in Figure 4 to Figure 7). This compatibility is vital across diverse tissue engineering applications, enabling these cell lines to thrive within the scaffold's microenvironment.

# Standard Operating Procedure

## Gelacell unpacking and sterilizing

1. Adhering to aseptic techniques, place the Gelacell™ package under a laminar flow hood and carefully cut the boundaries of the sterile pouch and remove the scaffolds. Place the scaffolds in a suitable cell culture vessel for in vitro growth and maintenance of cells.
2. The production of Gelacell takes place in clean rooms ISO Class 7 conditions and the products are sterilized by Gamma irradiation. UV sterilization for 20 minutes is advisable to retain sterility of the product upon unpacking.

**Note**: The scaffold can be either used in its present format or it can be cut into desirable shapes and sizes depending upon the experimental design and setups. It can also be used in a perfusion bioreactor or fixed in a cell culture inserts e.g. Cell Crown from Scaffdex. The scaffolds are expected to remain unchanged under standard UV-C radiation dosage. Higher irradiation doses should be used with caution.

## Cell seeding and culture

1. Before cell seeding, rinse the PLLA scaffold once with 70% ethanol, followed by a rinse with PBS solution three times. The volume of ethanol and PBS depends upon the usage of the scaffold. Ideally, the scaffolds need to be completely filled with ethanol and then use an excess amount of PBS for this purpose.
2. Pre-swell the scaffold with your preferred cell culture media (with/without serum). Add a desirable amount of media onto the scaffold, incubate for 30 min to 1 hour, and aspirate the spent media.
3. After pre-swelling, allow the scaffold to partially dry in a laminar hood by keeping the lid of the vessel open for 20 minutes. It helps in absorbing the cell seeding volume and spreading the cells uniformly.
4. Dispense your desired concentration of cell culture suspension with complete media onto the scaffold. The recommended cell seeding density ranges from $10^3$ to $10^5$ cells/$cm^2$, but the exact figure will depend on the cell type and the planned duration of cell culture.
5. Place the cell culture vessels in a CO2-incubator at 37°C for 30 minutes - 2 hours to allow initial cell adhesion. Afterward, gently fill the vessels with the medium without dislodging the cells that have already adhered to the scaffold. Return the scaffolds to the incubator for culturing cells.
6. It's recommended to exchange media roughly every 24 hours - 48 hours; however, this may vary depending on the cell line, media, and cell density. Aspirate the waste media from the sides of the vessels and carefully add fresh media on top of the scaffold without dislodging the adhered cells. Continue media exchange throughout the cell culture period.

**Note**: The cell culture vessels refers to the container where the scaffolds are kept for culturing cells such as, Petri dish, multiwell plates, bioreactors, microfluidic devices, culture flask, etc. The rate and efficiency of cell attachment and detachment can be affected by temperature, pH, nutrient exchange, the concentration of cells, enzymatic degradation, and cell staining. The above guidelines are the best-known practices based on the tests carried out on the scaffolds. The cell seeding volume is preferred to be in between 10 to 70 µm to achieve total cell adhesion and avoid cell losses. However, this seeding volume is not restricted for lower (< $10^4$) or higher (> $10^6$) seeding density, except the performance of cell adhesion might differ.

## Cell culture analysis

1. **[Optional] Cell fixation:** Rinse the scaffolds with PBS and use 3.7% - 4% formaldehyde or paraformaldehyde for cell fixation. Leave the scaffolds in the solutions for 15 min at room temperature or in a CO2-incubator.
2. **For cell viability:** Use assay tests such as MTS, MTT, CCK-8, etc. Follow the standard protocols suggested by the manufacturer. Ensure that the volume of the assay solution is maintained at a level three times the thickness of the scaffold.
3. **For cell staining and imaging:** Utilize stains such as Calcein, FDA, Phalloidin-conjugates, DAPI, or propidium iodide individually or combined to enhance contrast during imaging. Transferring the scaffolds to a glass slide can significantly improve cell visualization. Ensure that the volume of the staining solution is maintained at a level three times the thickness of the scaffold.
4. **Handling:** Use fine point tweezers/forceps (straight or angled) to hold the scaffold, and place it on a glass slide. Add one or two drops of mounting media to fix the inserts and cover it with a cover slip.
5. **Imaging:** Evaluate the scaffolds under a fluorescence or confocal microscope for imaging and visualization.

**Note**: The method of cell/tissue culture analysis on the scaffold may vary based on the tests and applications. The above guidelines are based on the tests conducted on the scaffolds. However, users are advised to extend the scope of analysis as per their requirements.

**Important Information:**

- The scaffold is compatible with both serum and serum-free cultures.
- The scaffolds are highly flexible (in some cases pliable) therefore, carefully grip the edges of the scaffolds when holding or transferring in the cell culture vessels to prevent flipping the scaffolds. Avoid forceful pipetting.
- Avoid scratching the scaffold while using micropipettes. When pipetting solutions from or into the cell culture vessel, place the tip at the vessel's periphery to avoid contact with the scaffold.
- The product maintains sterility until the package is opened, with a shelf life of 24 months from the dispatch date. Both sterility and shelf life are preserved when the product is stored according to the specifications outlined in the Safety Data Sheet.