



HYDROBIO  X400

“Cell encapsulation with subcellular precision!”



HYDROBIO INX[®] X400 is a gelatin-based hydrogel which provides all the biological benefits of gelatin, as an excellent extracellular matrix mimic, in combination with efficient high resolution processability thanks to a high reactivity and fast curing speed. Additionally, its excellent biocompatibility allows both cell encapsulation with high viability during printing as well as cell seeding after printing.

HYDROBIO INX[®] X400 is based on gelatin type B derived from natural collagen which is modified with photo-crosslinkable functional groups enabling efficient multiphoton processing at 780 nm in the presence of the supplied crosslinker.

After processing, it mimics the natural extracellular matrix because it is derived from natural collagen. Additionally, as the formed hydrogel is enzymatically biodegradable, it allows cells to remodel the environment and substitute it with newly formed extracellular matrix over time.

BIOLOGICAL APPLICATIONS

HYDROBIO INX[®] X400 is gelatin-based, making it suitable for a very wide range of biological applications. HYDROBIO INX[®] X400 allows the printing of biodegradable and soft hydrogel scaffolds with high resolution (i.e. down to 1 μm) for both cell seeding and cell encapsulation (see Figure 1). As a result, it opens new possibilities for researchers to include cells during and after printing to generate more complex constructs which mimic the natural 3D microenvironment found in native tissues.

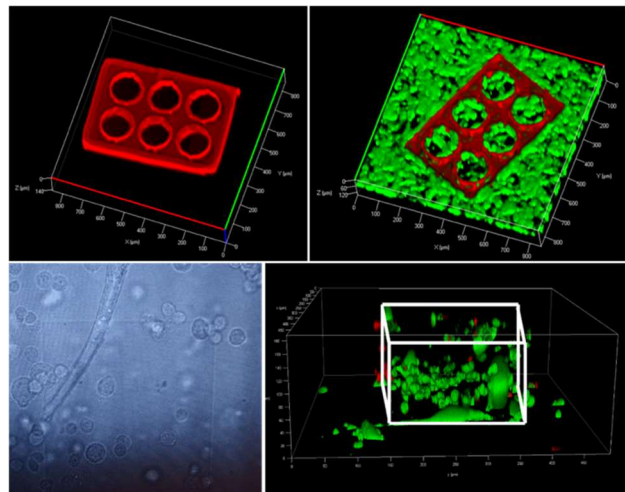


Figure 1: **Top Left:** HYDROBIO INX[®] X400 scaffold, **Top Right:** HYDROBIO INX[®] X400 seeded with HT1080 cells, **Bottom Left:** human foreskin fibroblasts (HFFs) encapsulated in HYDROBIO INX[®] X400 during printing on a Nanoscribe Photonic Professional GT 2; **Bottom Right:** encapsulated HFF's after 1 week of cell culture.



Examples of the biological performance of the HYDROBIO INX[®] X400 product family include:

- ✓ Cell seeding of human cells on 3D scaffolds
- ✓ Encapsulation of human foreskin fibroblast cells in HYDROBIO INX[®] X400 with high viability (>70%) both using UV or multiphoton lithography encapsulation

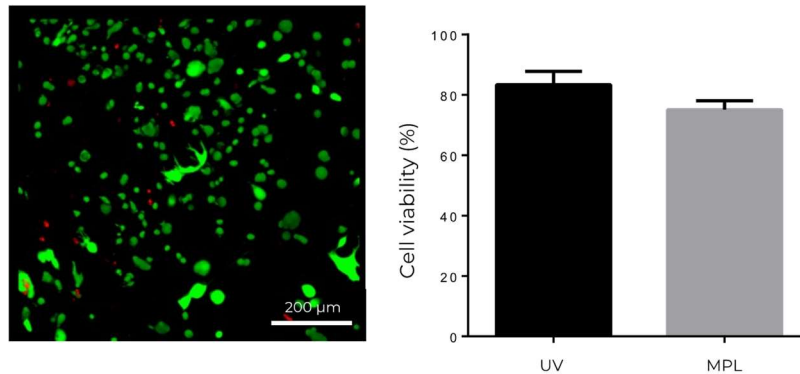


Figure 2: **Left:** HT1080 fibrosarcoma cells UV-encapsulated in HYDROBIO INX[®] X400. **Right:** Observed cell viability of encapsulated cells after 1 week via UV (fibrosarcoma cells) and multiphoton lithography (fibroblasts) based encapsulation.

PROPERTIES & PROCESSING

The HYDROBIO INX[®] X400 product family is offered as a 3-component system. HYDROBIO INX[®] X400 contains a red gel resin, 10 vials of crosslinker and a buffer. After heating, the red gel becomes liquid and can be diluted either with buffer or with an appropriate cell suspension in cell culture medium, if cell encapsulation is desired. After combining with the crosslinker, the material can be processed and will turn into a physical gel upon cooling, thereby providing support to free standing structures during printing and improving the mechanical properties after printing.

Physical Properties	HYDROBIO INX [®] X400
Appearance	Red gel
Storage Modulus (kPa)	2- 18
Post processing linear swelling (%)	10 - 30
Post processing total volume increase (%)	50 - 250



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HYDROBIO INX[®] X400 resins are characterized by fast curing kinetics, after which the material becomes physiologically stable (Figure 3). However, despite being physiologically stable, the material can still be degraded enzymatically by cells. After curing, the resin is characterised by a storage modulus in the range of 2 - 18 kPa, making it suitable for a range of soft tissue applications.

After processing, the material will take up a limited amount of medium, resulting in a small increase in dimensions after printing, typically ranging around a 10 - 30 % linear increase.

Additionally, HYDROBIO INX[®] X400 is multiphoton lithography-based photodegradable as well. After structuring softer regions or even channels can be generated by scanning a second time over the printed structure, thereby allowing for channel formation, cellular guidance, ... which leads to additional architectural complexity.

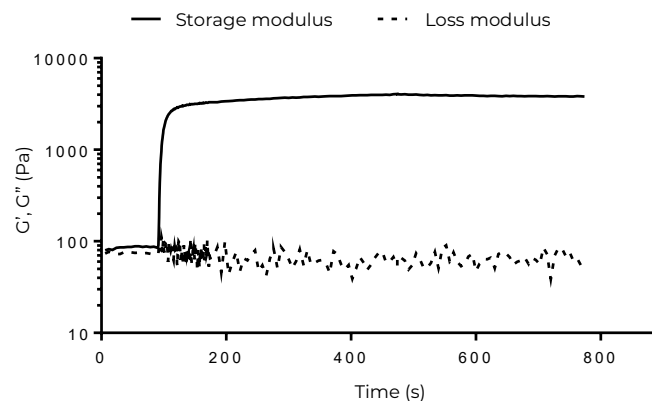


Figure 3: Storage and Loss modulus of HYDROBIO INX[®] X400 during UV crosslinking.

BENEFITS OF THE HYDROBIO INX[®] X400 PRODUCT FAMILY

- ✓ Biocompatibility: Supports cell adhesion and proliferation and allows for cell encapsulation
- ✓ Reproducibility: Production under strict quality control
- ✓ Biodegradability: Enables cellular remodelling of the printed matrix
- ✓ Photodegradability: Enables multiphoton induced photocleavage after printing
- ✓ Easy handling: Delivered in a ready-to-use kit containing a concentrated stock solution, dilution buffer and crosslinker for 10 prints in the presence or absence of cells. Ready for printing in 10 min
- ✓ Stable for at least 3 months at 4 - 8°C



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HYDROBIO INX[®] X400 combines all the benefits of conventional Gel-MA based bioinks with efficient high-resolution processing including cell encapsulation. As a consequence, it is the ideal material to transfer previous low-resolution Gel-MA related successes towards high-resolution applications.

Table 1: Main overview of the key features of HYDROBIO INX[®] X400 in relation to Gel-MA or HYDROBIO INX[®] X100.

	Conventional Gel-MA based Bioink	HYDROBIO INX [®] X100	HYDROBIO INX [®] X400
High resolution	✘	✔	✔
Cell-encapsulation	✔	✘	✔
Biodegradability	✔	✔	✔
Biocompatibility	✔	✔	✔
Hydrogel	✔	✔	✔
High reactivity	✘	✔	✔

3D PRINTER COMPATIBILITY

HYDROBIO INX[®] X400 can be used with a range of multiphoton lithography systems. It has been validated on the following multiphoton lithography systems:

- ✔ Nanoscribe Photonic Professional GT2
- ✔ UpNano NanoOne Bio
- ✔ UpNano NanoOne

If you would like to discuss your printer's compatibility with our resins, please contact us at info@bioinx.com