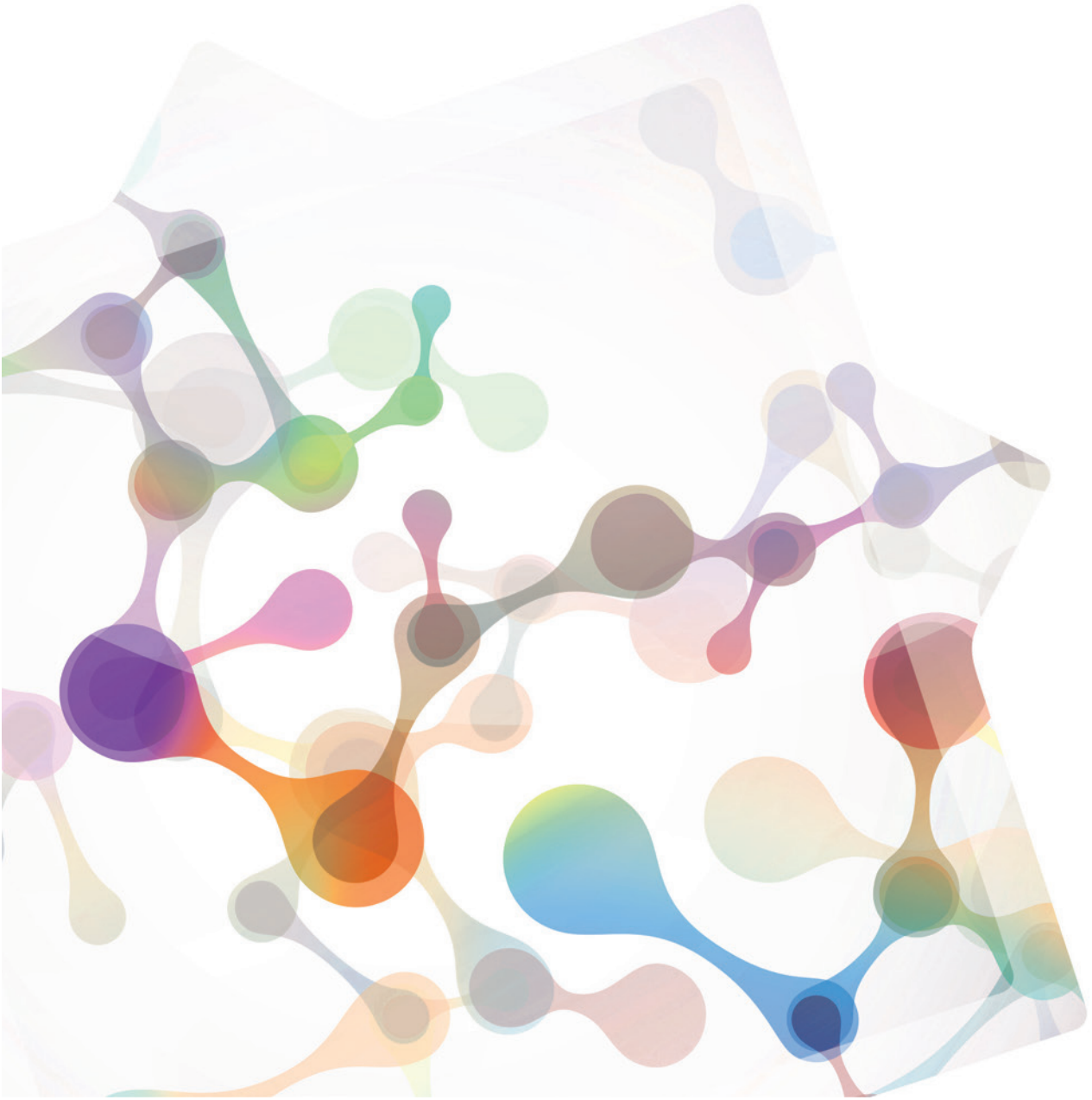
BIOINK SELECTION GUIDE

THE FUTURE OF MEDICINE IS HERE.



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WE ARE CELLINK

CELLINK is a global leader in developing and delivering life-science solutions, equipping hundreds of labs and thousands of scientists worldwide with cutting-edge technologies that fuel groundbreaking scientific breakthroughs. With a commitment to quality and innovation, our bioprinters, imaging systems and bioinks have contributed to revolutionary advancements in academic and clinical medicine.

CELLINK's solutions advance and accelerate the discovery process for customers in more than 60 countries, including university, hospital, pharmaceutical, public and commercial laboratories. Our domain knowledge, dedication to innovation and passion for excellence has positioned us as the driving market force in the fields of bioprinting and 3D cell culturing. We strive to push the limits of what is possible in regenerative medicine and pharmaceutical development, inspiring and empowering collaborators to expand their horizons and overcome any challenge they face. Join us on our journey to change the future of medicine: www.cellink.com.

COMMITMENT TO QUALITY

The best bioinks come from the best sources of raw materials. We select suppliers based on our strict standards to obtain the finest raw ingredients and deliver the maximum value possible to our customers. Once the raw materials are approved, they enter our sterile and toxin-free production process. We understand the importance of consistency in your research, and each final product undergoes stringent quality control to guarantee reproducibility. We verify bioinks' appearance, sterility, endotoxin level, cell viability, pH, viscosity, and, if required, functional characteristics like degree of methacrylation and gelation (Table 1).

Table 1. The properties and specifications of bioinks tested under quality control.

Property	Specification
Appearance	Translucent liquid/gel (*A Series*, *Coll 1*) Semi-translucent liquid/gel (*GelMA Series*, *GelX Series*, *ColMA*) Opaque gel (*CELLINK Series*)
Sterility	Free of bacteria, fungi and yeast
Endotoxin level	<10 EU/mL (*A Series*) <20 EU/mL (*Collagen Series*) <40 EU/mL (*CELLINK Series*) <50 EU/mL (*GelMA Series*, *GelX Series*) (Limulus Amoebocyte Lysate assay)
Cell viability	≥75% live cells (mesenchymal stem cells for 7 days)
pH	6.5-7.4 (for Collagen Series — after neutralization) (pH-meter)
Viscosity	Shear-thinning behavior (flow sweep in the shear rate range of 0.002-500 s^{-1}, at recommended printing temperatures)
Degree of methacrylation	45-55% (*GelMA Series*, *GelX Series*) 15-25% (*ColMA*) (^{1}H NMR performed at room temperature)
Gel point	24-28 degrees Celsius) (temperature sweeps in between 20-32 degrees at 1% strain and 10 rad/s angular frequency)
Thermal gelation	Gelling at 37 degrees when neutralized (*Coll 1*)

Sterility

We perform sterility testing to confirm that bioinks designed for studying cells are free of viable contaminative microorganisms. Our tests detect aerobic and anaerobic microorganisms at ambient and physiological conditions, including fungi and yeast.

Endotoxin level

Endotoxins are contained in the amphiphilic membranes of gram-negative bacteria. They can affect the functionality of human cells by altering their morphology and even destroying their membranes, especially in serum-containing cell culture media. Endotoxin sensitivity and acceptable endotoxin levels vary for different cell types.

pH

Intracellular pH is closely regulated in eukaryotic cells, and the pH of the external environment has a multifaceted impact on cell behavior, including effects on cell growth, essential metabolism and cell survival. For example, a pH above 7.4 causes cell cytoplasm contraction, and a pH below 6.5 irreversibly stops cell activity.

Cell viability

Our bioinks contain no cytotoxins. To ensure this, we test cell viability after seven days of culture in a bioprinted construct. We confirm that the cells grow and proliferate in the bioink environment.

Viscosity

Bioinks are often non-Newtonian fluids which demonstrate shear thinning — their viscosity decreases as shear stress increases. We verify that each bioink is shear thinning at given conditions to ensure your bioprinting is smooth and reproducible.

Degree of methacrylation

Controlling the degree of methacrylation ensures consistent photoinitiator-activated crosslinking through methacrylic functional groups. Crosslinking density has an impact on cell behavior because it defines mechanical properties like stiffness and swelling.

Thermal gelation

Upon heating, Coll 1 self-assembles into a gel with a much higher viscosity. This thermal gelation is crucial for collagen-based biomaterials as it keeps printed constructs together.

Gel point

GelMA-based bioinks have a distinctive gel point – the specific temperature that transforms a fluid bioink into a gel with a much higher viscosity. Understanding gel point is crucial for using GelMA-based bioinks, as the printing should be performed at the temperature just above it to minimize unfavorable flow (if temperature is too high) as well as filament breaking and nozzle clogging (if temperature is too low).

THE CELLINK SERIES STORY

FLAGSHIP BIOINKS FOR INNOVATIVE RESEARCH

Back in 2016, we at CELLINK recognized the need for a universal bioink formulation to advance the bioprinting field forward. As a result, we developed CELLINK Bioink as the first universal bioink for use in any bioprinting system, enabling convenient 3D tissue engineering with any cell type. This bioink is based on nanofibrillar cellulose and alginate derived from natural sources and developed under stringent quality control, ensuring consistency across your research experiments. Beyond offering excellent printability and cell viability, the bioink can be modified with a myriad of peptides and growth factors.

We developed a series of bioinks based on the CELLINK Bioink with the addition of RGD peptides, fibrinogen, tricalcium phosphate and laminins. We are proud to show you the best examples of using CELLINK Bioink as the foundation for biomedical applications. Simply mix CELLINK Bioink with a high concentration of cells and bioprint your desired structure. Crosslink with our $CaCl_2$-containing Crosslinking Agent, wash and culture in your desired cell medium.

To learn more about the CELLINK series or get a quote, visit www.cellink.com/bioinks/cellink-series. You can also scan the QR code with your smartphone to go straight to our website.

UNIQUE BIOINK CHARACTERISTICS

Combining the advantages of shear-thinning nanofibrillar cellulose and the versatility of alginate, CELLINK Bioink has unparalleled consistency and printability (Figure 1). CELLINK Bioink has a smooth flow at a wide range of temperatures and maintains its shape after printing (Figure 2A).

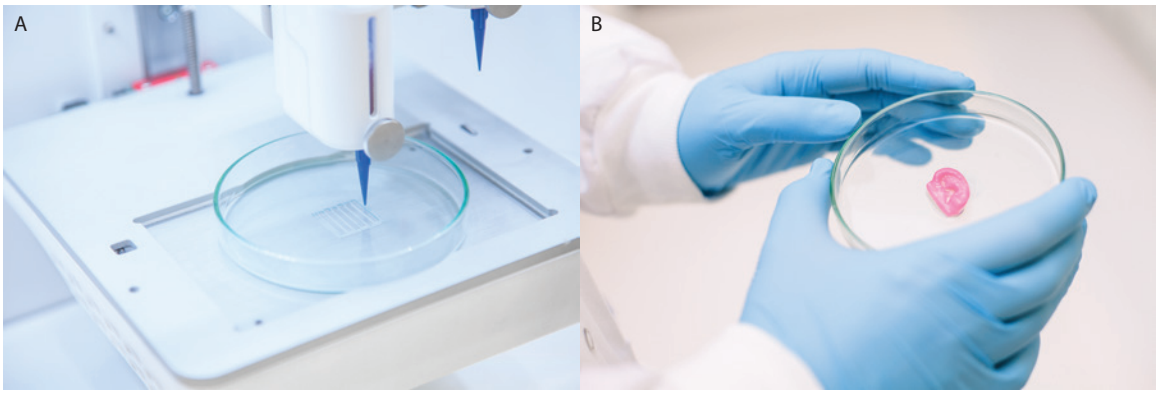


Figure 1. 3D-printed constructs with CELLINK Bioink using the BIO X system. A) Grid. B) Ear.

Well-dispersed cellulose nanofibrils enable stable CELLINK bioinks with shear-thinning behaviour perfect for bioprinting, as their viscosity decreases consistently with increased shear rate (Figure 2B). The CELLINK LAMININKs have a slightly lower viscosity to form softer 3D-bioprinted constructs.

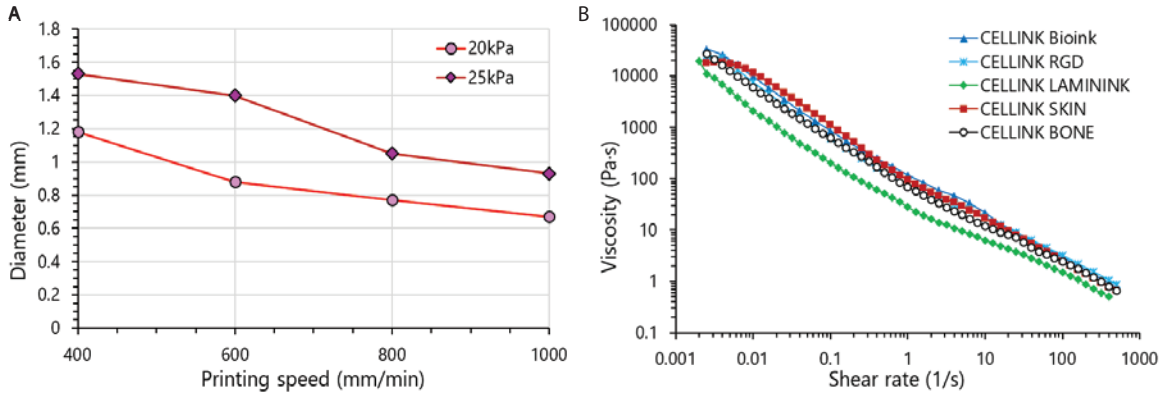


Figure 2. A) Filament diameter dependence on printing speed for CELLINK Bioink using 25G nozzle at 20 and 25 kPa.
B) Viscosity dependence on shear rate for the CELLINK series.

CELLINK® Bioink

Description

CELLINK Bioink is the first universal bioink designed to optimize 3D bioprinting of human tissues. CELLINK Bioink is a nonanimal derived polysaccharide hydrogel comprised of a cellulose and alginate base. Its biocompatibility and ease-of-use make it an excellent bioink for 3D cell culturing. Early research with CELLINK Bioink showed the bioink's capacity to support the synthesis of cartilage-specific extracellular matrix components by human chondrocytes. Additionally, CELLINK Bioink demonstrated prolific compatibility with skin and tumor tissues.

Research application

CELLINK Bioink was used for cartilage regeneration in Nguyen et al., where 3D-bioprinted constructs laden with iPSC-derived chondrocytes were cultured for five weeks. In Figure 3A, immunostaining reveals type II collagen production by the iPSC-derived chondrocytes. In Figure 3B, a tissue stained with Alcian blue and van Gieson's dye (A&G) for glycosaminoglycans (blue) and cells (pink), shows well-distributed chondrocytes with rounded morphology throughout the 3D-bioprinted construct after only three weeks. (Nguyen et al. Scientific Reports, April 2017.)

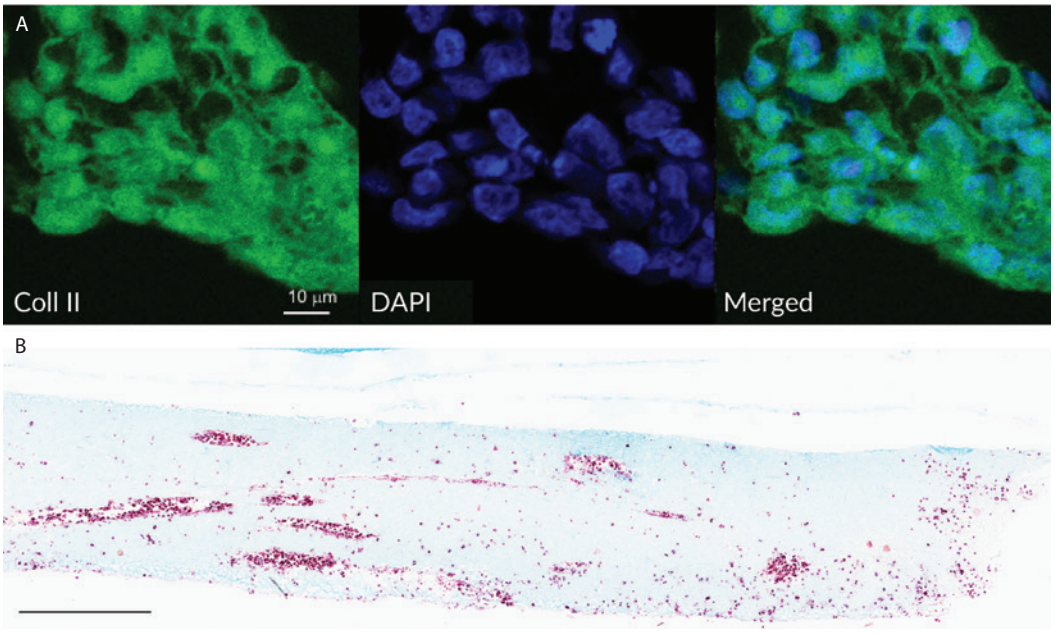


Figure 3. A) Immunostaining of chondrocytes within the 3D-bioprinted constructs after five weeks of culture with positive type II collagen staining (green) and cell nuclei (blue). Scale bar: 10 µm. B) A&G staining of chondrocyte clusters after three weeks in CELLINK Bioink. Scale bar: 500 µm.

CELLINK® RGD

Description

CELLINK RGD is a modified composition of CELLINK Bioink. This bioink contains alginate covalently conjugated with the cell attachment peptide sequence RGD. The sequence is derived from fibronectin and is the most commonly recognized sequence by cells for adhesion. RGD has wide applicability for engineering nearly every tissue. The sequence has also been used for the surface treatment of bone and vascular implants to improve integration in the body.

Research application

CELLINK's scientists printed CELLINK RGD with fibroblasts. We used Haematoxylin and Eosin (H&E) staining to visualize cell nuclei (purple) and the production of type I collagen and extracellular matrix (light pink). H&E staining indicated production of extracellular matrix components and fibroblasts distributed throughout the bioink at day 14 (Figure 4).

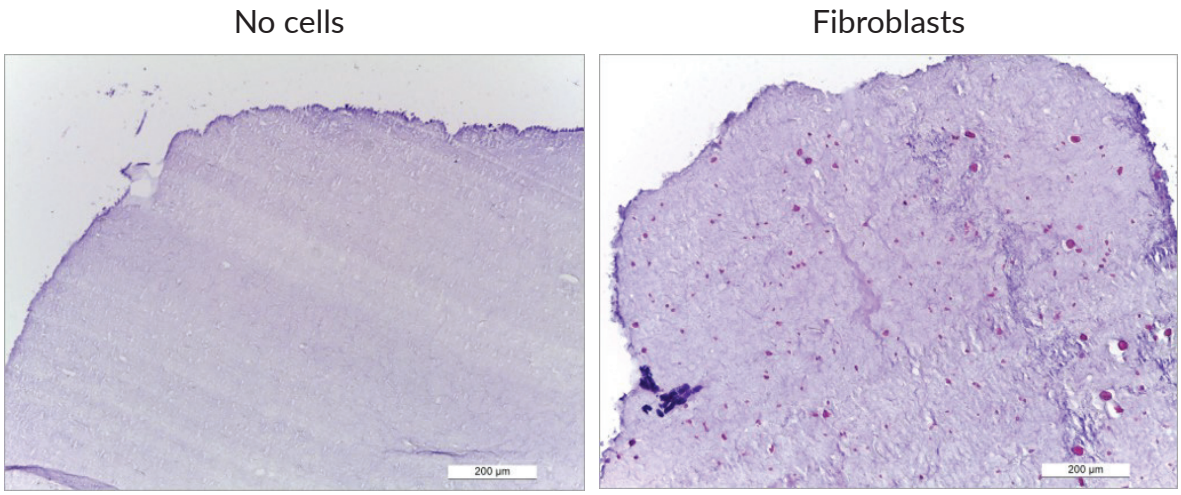


Figure 4. H&E staining of 3D-bioprinted constructs without cells and with fibroblasts in CELLINK RGD at day 14. Scale bars: 200 µm.

CELLINK® FIBRIN

Description

CELLINK FIBRIN was developed for fabricating many different tissues and skin-related constructs for wound healing. Fibrin is a protein critical in tissue healing due to its clotting capabilities. CELLINK FIBRIN comes with a crosslinking solution enhanced with thrombin to enable development of a stable network. After crosslinking with thrombin, CELLINK FIBRIN contains both *in situ* fibrin and fibrinogen to provide a more physiological wound-healing environment.

Research application

Our customers at the University of Pavia bioprinted C2C12 myoblasts in CELLINK FIBRIN to study cell differentiation and muscle fiber physiology. The cells survived, differentiated and demonstrated myotube formation at day 7 (Figure 5A). Actin and myosin formation, as well as expression of two important master genes related to muscle differentiation (MyOD and MCK), demonstrate the positive effect of the 3D construct on the differentiation process (Figure 5B). (CompMech Group and Regenerative Medicine Lab, University of Pavia.)

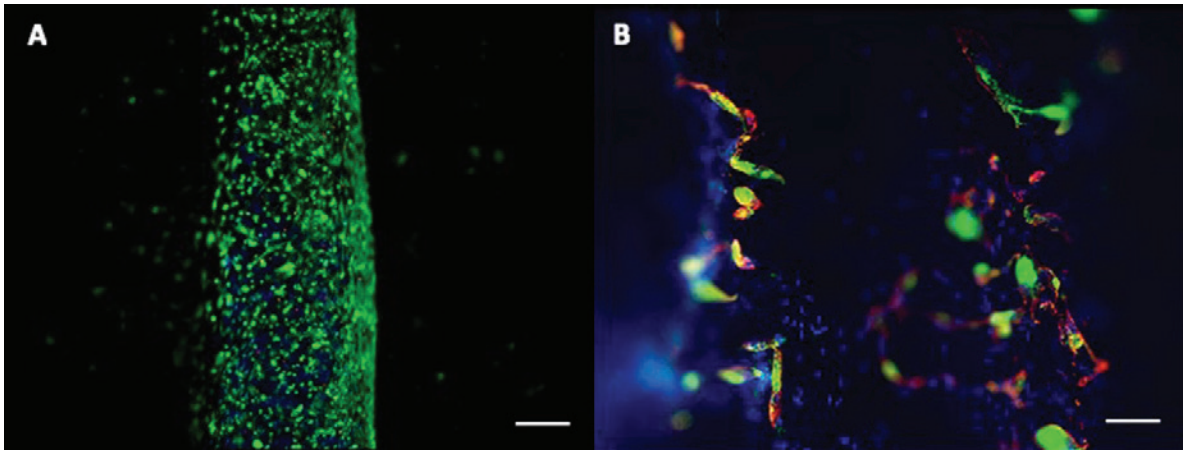


Figure 5. A) Live/dead assay of myoblasts laden in CELLINK FIBRIN bioink in proliferative condition after seven days of culture. Green indicates live cells and red indicates cells in apoptosis. Scale bar: 50 µm. B) Immunofluorescence analysis after 27 days of differentiation. Green indicates actin filaments stained by Phalloidin and red indicates myosin filaments MF20 positive. Scale bar: 100 µm.

CELLINK® SKIN

Description

CELLINK SKIN is optimized for culturing fibroblasts, keratinocytes and melanocytes for skin and skin-related constructs. This bioink contains fibrinogen to recreate the native wound-healing environment. CELLINK SKIN comes with a crosslinking solution enhanced with thrombin to support the formation of a full-thickness skin tissue model. After crosslinking with thrombin, CELLINK SKIN contains *in situ* fibrin and fibrinogen to provide an environment that mimics the native wound-healing process. CELLINK SKIN is perfectly adapted for skin model research and compound testing.

Research application

CELLINK scientists used CELLINK SKIN to bioprint a full-thickness skin tissue model with dermis and epidermis layers. CELLINK SKIN supports cellular proliferation — cell number visibly increased between day five and 14 — as well as cellular alignment of fibroblasts. ECM fibers can be seen in red at day five and less visibly at day 14 due to high cellular interference (Figure 6).

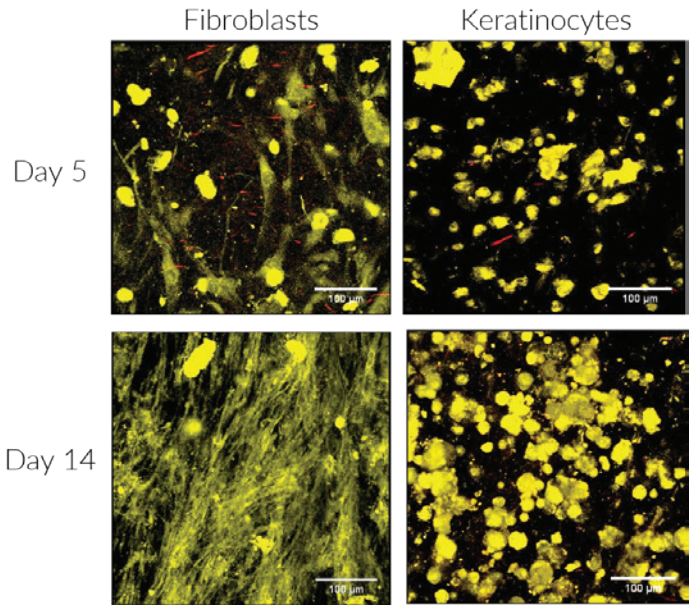


Figure 6. Multiphoton images of fibroblasts and keratinocytes bioprinted in CELLINK SKIN at days 5 and 14. Yellow: cells. Red: ECM. Scale bars: 100 µm.

CELLINK® BONE

Description

CELLINK BONE is a modified composition of our universal CELLINK Bioink designed to guide cell differentiation and impart osteogenic capacity. Tricalcium phosphate particles are uniformly dispersed within CELLINK BONE to ensure consistent printability. CELLINK BONE is intended for use with cell types and tissue applications related to bones, joints and their interfaces, such as the bone-to-ligament or bone-to-cartilage transition regions. CELLINK BONE can be used as a base material for fabricating bone constructs and it can be supplemented with other osteogenic factors like BMP-2 to further enhance its properties.

Research application

Dr. Luis De Bellis and Dr. Juan Carlos Carvajal from the University of Chile used CELLINK BONE in an *in vivo* bone repair experiment. They used CELLINK BONE with a scaffold to encourage bone regeneration in a rat bone defect. The histological image shows the formation of a new bone in streaks of purple (Figure 7). The purple areas with more cell nuclei represent new bone formation and the pink areas represent a more mature bone.

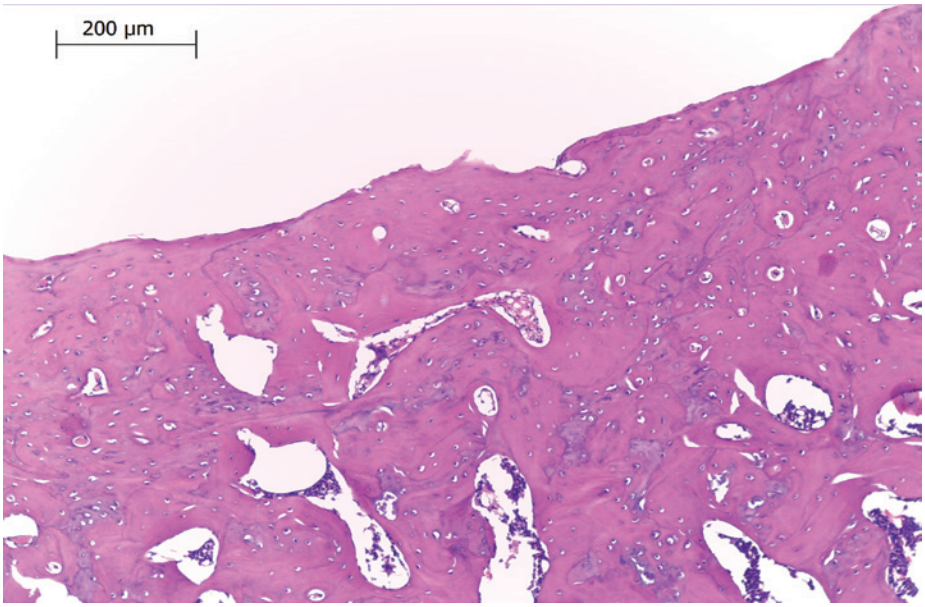


Figure 7. H&E histology image of bone formation in purple.

CELLINK® LAMININK

Description

The CELLINK LAMININKs include CELLINK LAMININK 111, 121, 411, 521 and +. These bioinks integrate different laminins to mimic the basal lamina and the natural tissue environment. Laminins play an important role in cell differentiation, migration and adhesion, and they also help support a healthy tissue. CELLINK LAMININKs are designed to improve tissue maintenance and survival. The bioinks maximize cell viability and are a great starting point for culturing many cell types. Compared to other heterogenous matrices, our laminins' xenographic source enables better control over the matrix environment.

Research application

To demonstrate CELLINK LAMININK 121's functionality, CELLINK's scientists bioprinted liver tissue models with Hep G2 cells (Figure 8A) and GFP-labelled iPSC-derived motor neurons in a custom CELLINK LAMININK blend (Figure 8B). Our customer Professor Cristina Scielzo at the San Raffaele Scientific Institute 3D printed primary leukemic cells in the CELLINK LAMININKs and analyzed their cross-talk with the microenvironment (Figure 8C).

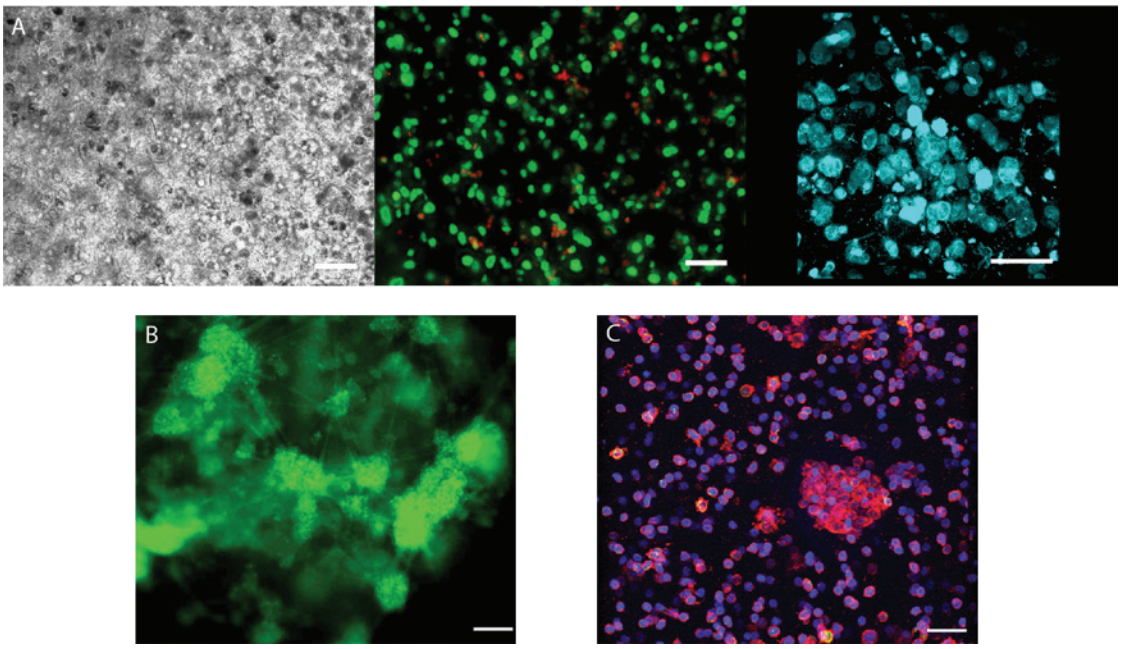


Figure 8. A) At day seven, the left photo is a brightfield image showing cells evenly distributed within the cell-laden CELLINK LAMININK 121. The center photo shows live/dead staining indicating minimal dead cells (red). The right photo is a label-free microscopy image of Hep G2 (cyan; multiphoton microscopy). Scale bars: 100 µm. B) Clustering of motor neurons at day 17. They develop a 3D neural network between individual clusters and form internal networks between individual cells. The image was captured using a fluorescent microscop. Scale bar: 200 µm. C) Confocal microscopy of 3D-bioprinted leukemic cells in CELLINK LAMININK 111 after 14 days of culture. Red: phalloidin staining. Blue: DAPI. Scale bar: 100 µm.

GelMA SERIES STORY

THE BIOINK WITH A CELL'S MENTALITY

Gelatin methacrylate (GelMA) has become one of the cornerstone bioinks in the bioprinting field due to its extraordinary versatility across applications. Labs all over the world can synthesize their own GelMA, which contributes to its popularity but also creates margins of inconsistency. This makes it difficult to standardize bioink formulations and reproduce experiments.

At CELLINK, we aim to provide the most consistent and highest quality bioinks. This approach extends to our GelMA bioink and the broader GelMA series. Through large-batch protection and stringent quality control, CELLINK delivers the most consistent GelMA on the market. This eliminates batch-to-batch variance and facilitates the development of standardized CELLINK GelMA formulations that act as a foundational bioink for countless research laboratories around the world.

What is GelMA?

GelMA is a photocrosslinkable version of gelatin with amine groups that are partially replaced with methacrylate groups. Photocrosslinking facilitates stability of printed constructs in cell culture conditions. The widespread use of GelMA hydrogels in the biomedical field is due to their unique biological properties that allow excellent attachment and proliferation of various cell types.

To learn more about the GelMA Series or get a quote, visit www.cellink.com/bioinks/gelma-series. You can also scan the QR code with your smartphone to go straight to our website.

UNIQUE BIOINK CHARACTERISTICS

Our GelMA's characterization starts with its printability and rheological properties. With CELLINK's GelMA, you can print a continuous filament with a ratio of filament diameter to inner nozzle diameter of 2.8-4.5 for a 27G nozzle (Figure 9).

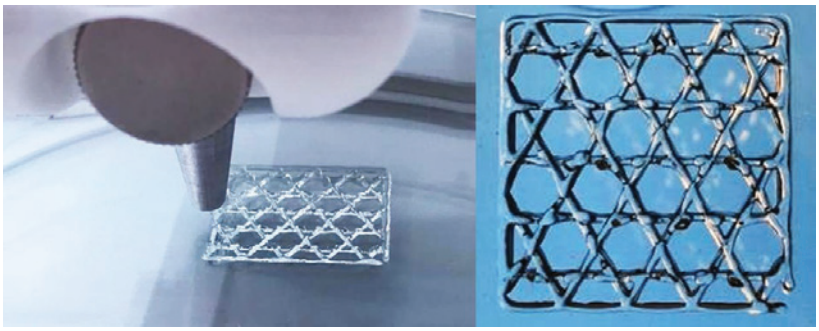


Figure 9. 3D bioprinted GelMA constructs.

For all bioinks, you can decrease the printing pressure and increase the printing speed to reduce the filament diameter. GelMA-based bioinks are dependent on the thermal environment during the printing process because they have a distinct gel point around 25-26 degrees Celsius. The gel point is defined as the intercept between the storage and loss moduli curves (Figure 10A). The GelMA Series bioinks are optimized for bioprinting near the gel point as their viscosity decreases steadily as the temperature rises (Figure 10B). The bioinks in the series have liquid-like behavior above the gel point and solid-like behavior below the gel point.

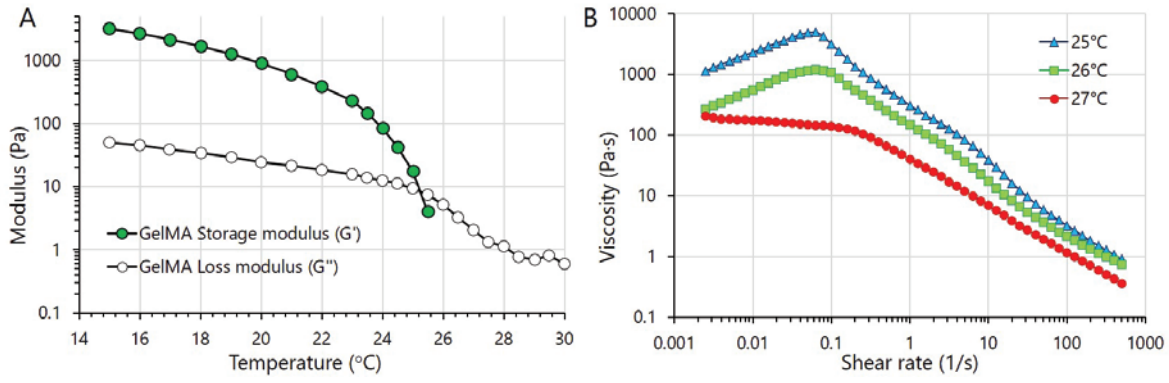


Figure 10. A) Storage and loss moduli dependence on temperature for GelMA. B) Viscosity dependence on shear rate for GelMA measured at different temperatures.

Bioprinted GelMA crosslinked easily through the photopolymerization of methacrylic functional groups initiated by the photoinitiator LAP. It takes only five seconds to obtain a mechanically stable construct, and users can increase the photocrosslinking time to achieve higher stiffness qualities (Figure 11A).

Due to their amorphous nature and the force needed to begin flow, all GelMA-based bioinks demonstrate shear thinning at shear rates above 0.1 s^{-1} (Figure 11B). GelMA A and GelMA HA have lower viscosities because they are blended with alginate and hyaluronic acid, respectively.

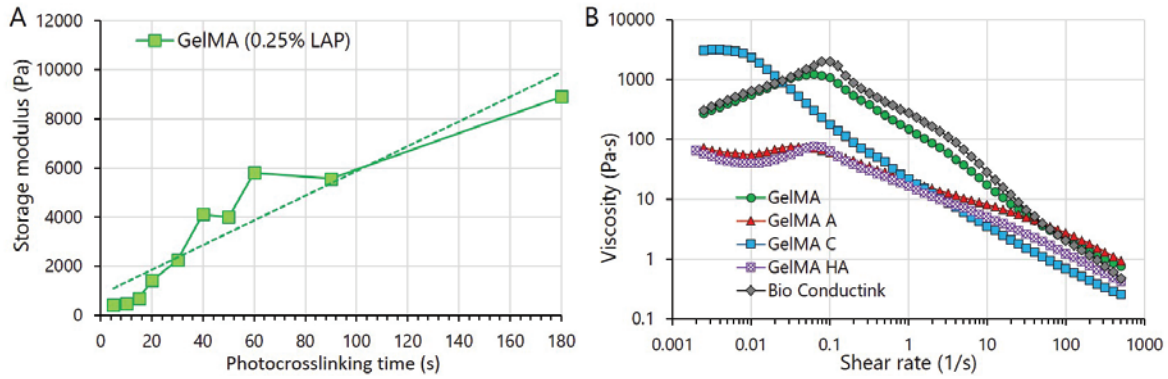


Figure 11. A) Storage modulus dependence on photocrosslinking time for GelMA. B) Viscosity dependence on shear rate for the GelMA series bioinks at 26 degrees Celsius.

Additionally, GelMA's miscibility with various value-added components creates limitless options for biomedical research. At CELLINK, we developed several special GelMA-based bioinks:

- GelMA A crosslinks in multiple ways to offer mechanical tunability.
- GelMA C provides printability and shear thinning at a wide range of temperatures.
- GelMA HA contains the ECM component essential for enhanced cell attachment and growth.
- Bio Conductink is a conductive bioink that enables electrical signals to transfer between cells.

GelMA

Description

CELLINK GelMA is compatible with most mammalian cells and can be used as a base material for a wide range of tissues. This bioink is designed to print with a temperature-controlled printhead and a cooled printbed. GelMA must be directly cooled after printing to maintain its shape before crosslinking. GelMA is very sensitive to the thermal environment during printing and the recommended printing temperature is 26 degrees Celsius.

Research application

To evaluate cell behavior in GelMA, we cultured adult human dermal fibroblasts (HDFs). HDFs generate connective tissue to help skin recover from injury. Bioprinted and photocrosslinked GelMA constructs create an ideal environment for cell adhesion and formation of an extensive network with well-stretched HDFs — their natural physiological shape (Figure 12A). Additionally, applying GelMA's biocompatibility and swelling properties can generate uniform concentric compression for different wound-healing tissue constructs in a process similar to the one shown in Figure 12B.

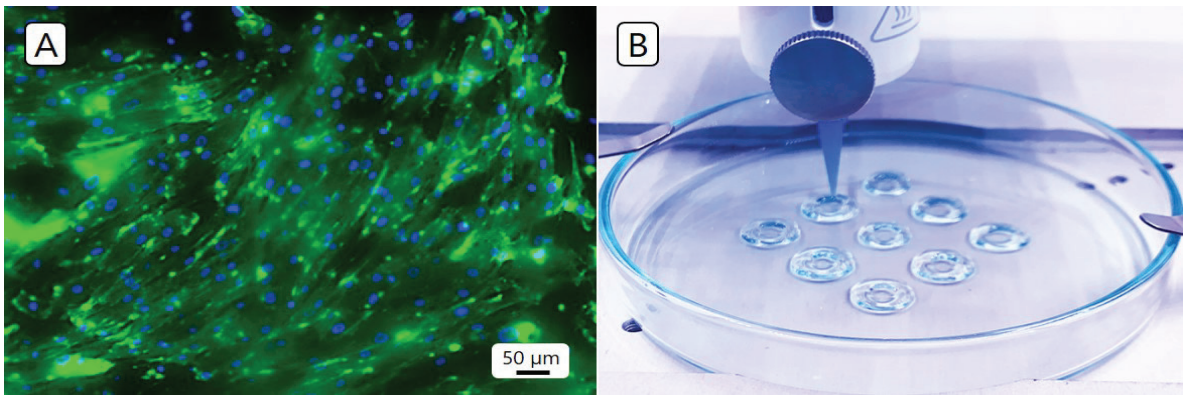


Figure 12. A) Fluorescence microscopy images of fibroblasts after 14 days of culturing in CELLINK GelMA. Cell nuclei are stained with DAPI (blue) and actin filaments are stained with Alexa Fluor® 488 phalloidin (green). B) Examples of rings for wound healing, 3D printed using a demonstrative bioink (A.J. Mellott, University of Kansas Medical Center).

GelMA A

Description

GelMA A combines the advantages of GelMA and alginate. This blend has excellent printability at room temperature and a minimal risk for nozzle clogging, even in bioprinting systems with no thermal control. You can crosslink GelMA A with a photoinitiator and 405-nm wavelength light, or by adding our Crosslinking Agent. Different crosslinking options enable you to develop constructs with mechanical properties that best match target tissues.

GelMA A is moderately sensitive to the thermal environment during printing. After a thermal reset, i.e. reheating of the bioink to adjust the chain entanglements, GelMA A prints easily at temperatures between 20 and 26 degrees Celsius. GelMA A's gel point cannot be clearly defined as it behaves similarly between 25 and 27 degrees (Figure 13).

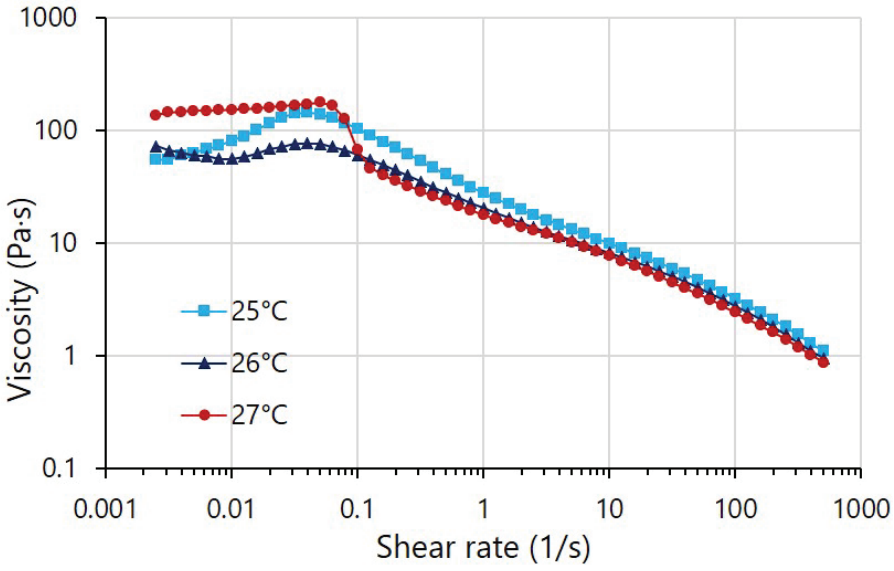


Figure 13. Viscosity dependence on shear rate for GelMA A measured at three temperatures near the gel point.

GelMA C

Description

GelMA C combines the advantages of GelMA and nanofibrillar cellulose. This blend has smooth printability at room temperature without thermal control and provides fibrillar morphology that benefits particular cell types, including muscle, skin, epithelial and neural cells. GelMA C crosslinks quickly through photoinitiator activation or through the addition of our ionic Crosslinking Agent. GelMA C is shear thinning in physiological conditions and maintains excellent printability at low extrusion pressures with a wide range of nozzle diameters.

Research application

We used GelMA C to bioprint hepatic LX-2 stellate cells — fibrogenic cells used to model chronic liver disease in humans, such as fibrosis. By day seven, the cells proliferated and clustered to form small spheroids which then further fused together in a larger structure by day 14 (Figure 14). Based on scientific literature, the bulky shape of the cells might indicate the retinoid-storing capacity of the presented hepatic cells.

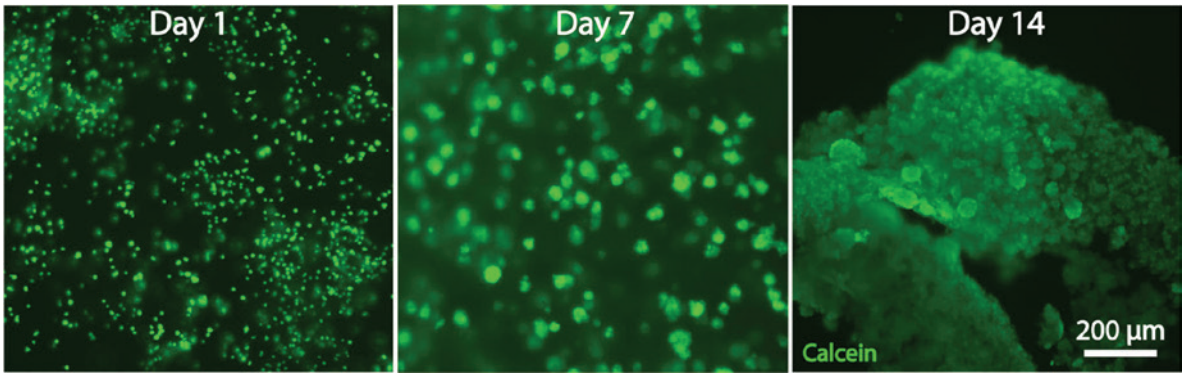


Figure 14. Fluorescence microscopy images of hepatic LX-2 stellate cells after one, seven and 14 days of culturing in GelMA C bioprinted using BIO X. Cells are stained with calcein. Scale bar: 200 µm for all images.

GelMA HA

Description

GelMA HA blends GelMA with methacrylated hyaluronic acid (HA). HA is a main component in the extracellular matrix of many tissues, including skin, cartilage, bone and nerves. It is essential for cell proliferation and migration and is an exciting material for biomedical research.

Research application

We tested GelMA HA for bioprinting using mesenchymal stem cells (MSCs) derived from bone marrow. MSCs are multipotent because they can develop into multiple specialized cell types depending on the surrounding environment. After seven days (Figure 15A), the MSCs showed stretched morphology and cell communication through long and extended cell processes, demonstrating that HA facilitates cell adhesion and growth. At day 14 (Figure 15B), the cells were aligned and stretched, demonstrating the bioink's biocompatibility.

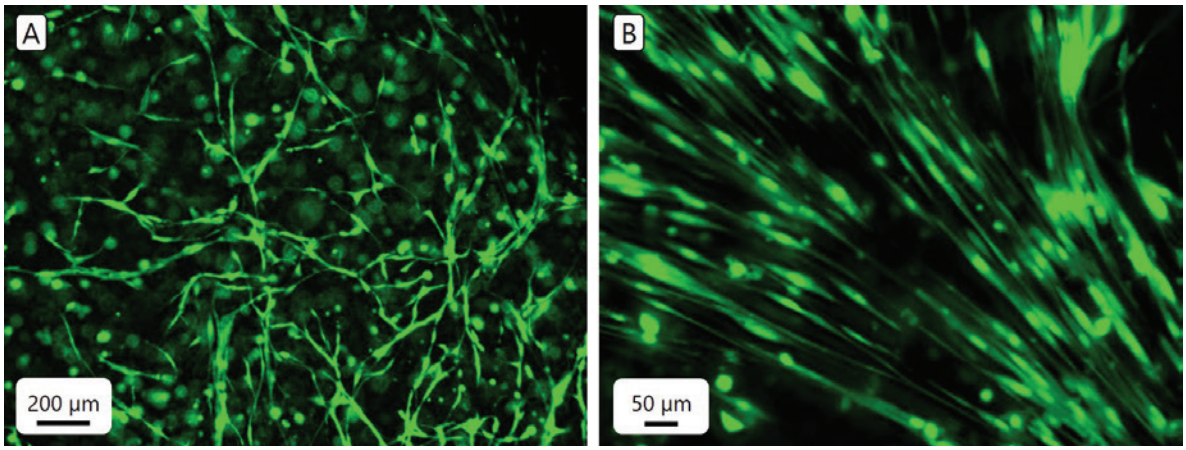


Figure 15. Fluorescence microscopy images of mesenchymal stem cells (MSCs) after (A) seven and (B) 14 days of culturing in GelMA HA bioprinted using BIO X. Cells are stained with calcein.

Bio Conductink

Description

Bio Conductink is designed for studying muscular contraction and nerve repair. Bio Conductink creates an electrically conductive cell environment for neural and muscle cells using electrically conductive additives such as carbon nanotubes (CNTs). This enables cells to transfer electrical signals, enhancing their communication and capacity for network formation. Bio Conductink leverages CNTs to give users excellent printability with low cytotoxicity, which is important to emphasize in the context of previously reported cytotoxic effects of different CNT-containing materials.

To demonstrate Bio Conductink's conductivity, we used it to conduct electrons throughout a 3D printed construct and create an electrical circuit to light an LED (Figure 16).

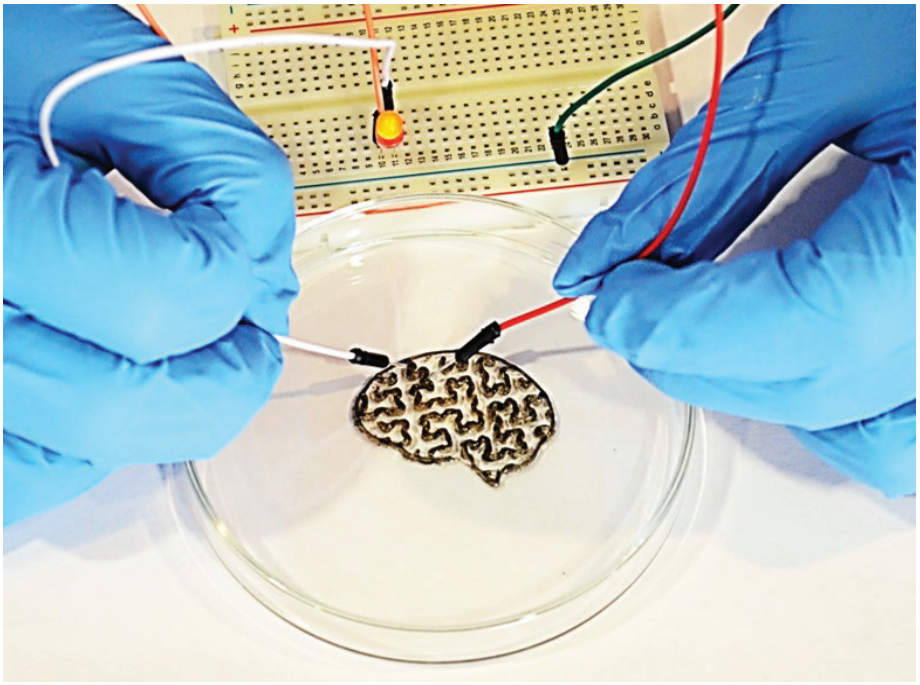


Figure 16. Using crosslinked Bio Conductink to complete an electrical circuit and light an LED.

GelX SERIES STORY

THE BIOINKS WITH DUAL ACTION AND VERSATILITY

At CELLINK, we formulate biomaterials into printable, crosslinkable and easy-to-use bioinks for all research applications to advance the bioprinting field. The GelX series leverages methacrylated gelatin to combine the biological characteristics of a crosslinkable gelatin network with printability at a wider range of temperatures. The bioinks' ease-of-use and versatility in crosslinking enable bioprinting of soft and hard tissue models. The series includes bioinks modified with tricalcium phosphate, laminins and fibrinogen to create favorable environments for all cell types including cell lines, stem cells and primary cells.

Our bioink development process includes stringent quality control and in-house research to optimize products and protocols for our customers.

Here, we present examples of bioprinting applications using the GelX series bioinks that can help inspire your next 3D tissue engineering experiment.

To learn more about the GelX series or get a quote, visit www.-cellink.com/global/bioinks/gelx-series. You can also scan the QR code with your smartphone to go straight to our website.

UNIQUE BIOINK CHARACTERISTICS

GelX-based bioinks have GelMA's biological properties and printability at a wide range of temperatures. Two culture conditions can be used when engineering tissues with GelX-based bioinks: the normal culture conditions of submerging the construct in medium (Figure 17A) and the phasic culture conditions of an air-liquid interface (Figure 17B), which can both influence epidermal/epithelial tissue models.

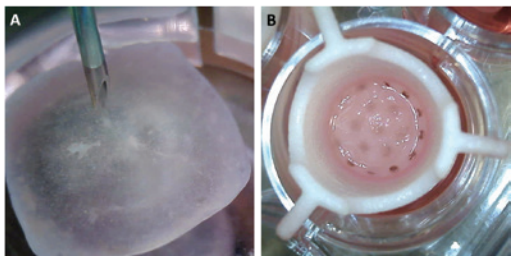


Figure 17. 3D bioprinted constructs using GelX series bionks to create tissue models for culture (A) submerged in medium or (B) at air-liquid interface.

The GelX series contains xanthan gum, which provides a stabilizing effect and enables a homogenous, printable hydrogel formulation at room temperature (Figure 18). For temperature-sensitive cells, users can bioprint at 37 degrees Celsius while retaining printing stability.

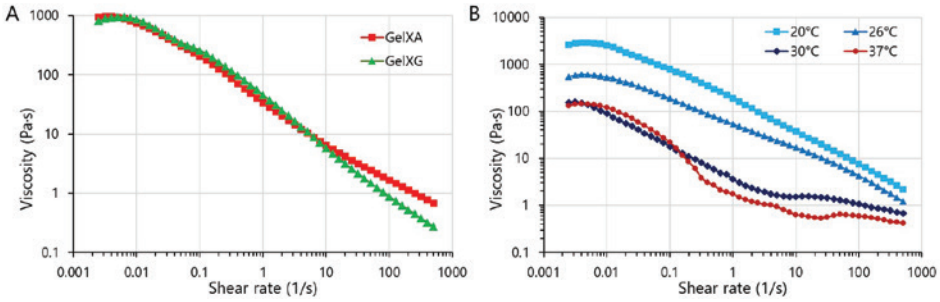


Figure 18. (A) Viscosity dependence on shear rate for GelXA and GelXG at 25 degrees Celsius. (B) Viscosity dependence on shear rate for GelXA measured at different temperatures.

Excluding GelXG, GelX-based bioinks support both ionic and photo-induced crosslinking to better accommodate cellular sensitivity and to tune each construct's mechanical characteristics to be more tissue specific. To support nondestructive analysis, GelXA bioinks have low optical interference in brightfield and fluorescent observation when ionically crosslinked. They also have low background effects in histochemistry analysis, providing the environment necessary to closely investigate cell-cell and cell-matrix interactions.

GelXG

Description

GelXG is a gelatin-based bioink that is designed to withstand a wider temperature range than GelMA-based bioinks. For cells with sensitivity to alginate, GelXG is a great photocurable alternative. GelXG does not have a distinct gel point; its storage and loss modulus curves do not intercept, providing elastic properties and reducing flow after bioprinting (Figure 19A).

Research application

GelXG is compatible with most mammalian cells. GelXG can be used to study how cells migrate as individual cells and as clusters, like in the lung adenocarcinoma model shown in Figure 9B. The 3D-bioprinted cancer model demonstrates that cells can form aggregates and migrate freely within photocured GelXG. Figure 19B shows cancer clusters and tunnels, demonstrating proof of migration.

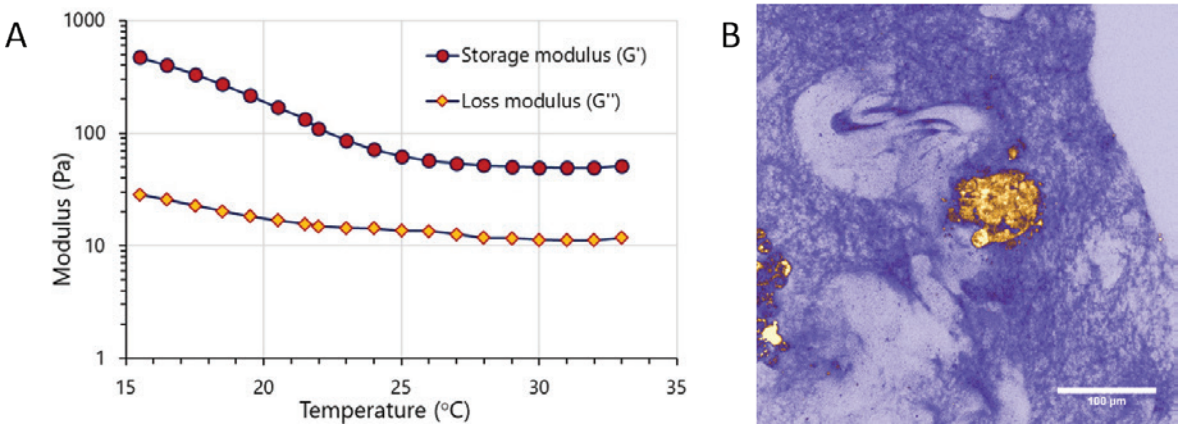


Figure 19. The GelXG bioink has a (A) moduli dependence on temperature. (B) 3D-bioprinted GelXG cancer tissue model observed with multiphoton microscopy shows a cancer cell cluster (yellow) migrating, leaving behind tunnels within the GelXG bioink (purple). Scale bar: 100 µm.

GelXA

Description

GelXA supports ionic and photo-induced crosslinking. Dual crosslinking capabilities make it possible to tune the construct's stiffness to the environment of a specific cell type (Figure 20). This enables researchers to analyze tissue-specific models to observe cell-matrix interactions and responses such as cell proliferation, differentiation and migration. Warming GelXA to 37 degrees Celsius liquifies the bioink and allows for gentle cell mixing.

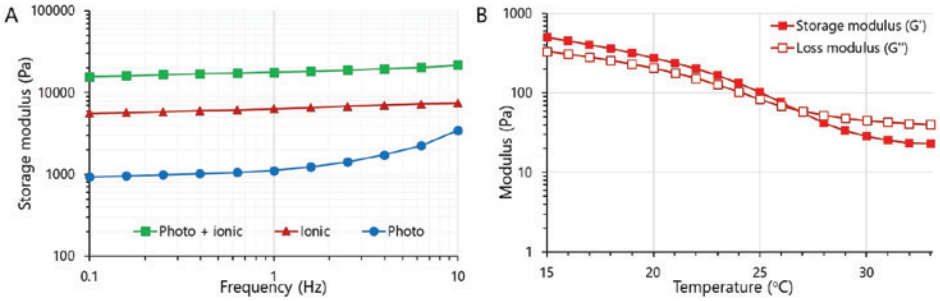


Figure 20. A) Frequency sweeps of photocrosslinked, ionically and dually crosslinked GelXA at 37 degrees Celsius; fixed amplitude of 1%; performed immediately after crosslinking. B) Storage and loss moduli dependence on temperature for GelXA.

Research application

The bioprinted LX2 cell-laden GelXA constructs demonstrate the influence of different crosslinking methods on cell proliferation patterns (Figure 21). With the addition of ionic Crosslinking Agent, the cells formed isolated clusters, while the cells in photocured constructs formed branching cell networks that were most evident at day seven. At day 14, cell viability was higher in photocured constructs.

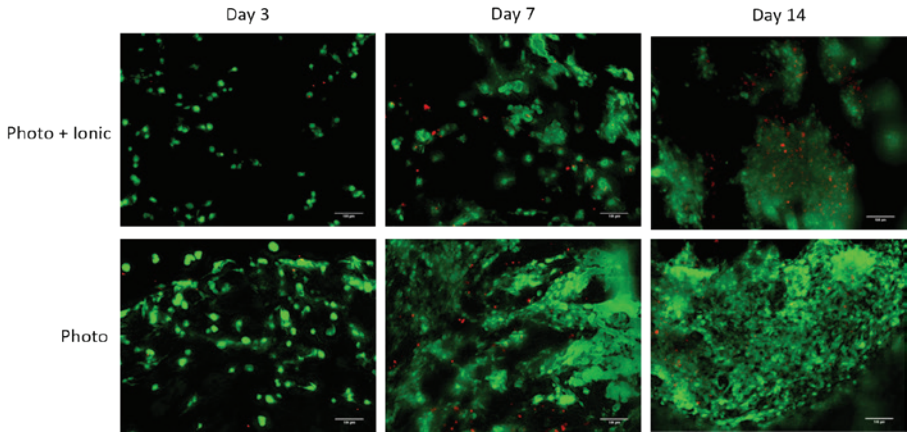


Figure 21. Microscopy images of live (green) and dead (red) LX2 cells in GelXA, photocured and ionically crosslinked and cultured for 14 days. Scale bars: 100 µm.

GelXA BONE

Description

GelXA BONE incorporates tricalcium phosphate and hydroxyapatite particles to create an osteoinductive-like environment. GelXA BONE constructs crosslink both through photocuring and through the addition of our ionic Crosslinking Agent. As bone cell differentiation depends on environmental stiffness, users can leverage different combinations of crosslinking and timing to tune GelXA BONE for the target bone or cartilage progenitor cells. GelXA BONE is tailored for tissue applications related to bones, joints and interfaces, like the bone-to-ligament and bone-to-cartilage transition regions. It is a ready-to-use base material for bone constructs and a complement for thermoplastic scaffolds such as PCL and PLGA.

Research application

To support cell viability, we tailored the bioprinting characteristics of GelXA BONE to enable printability at a wide range of temperatures as it has no distinct gel point (Figure 22A). At CELLINK, osteoblasts bioprinted in GelXA BONE showed stretched out morphology at day 35 of culture (Figure 22B).

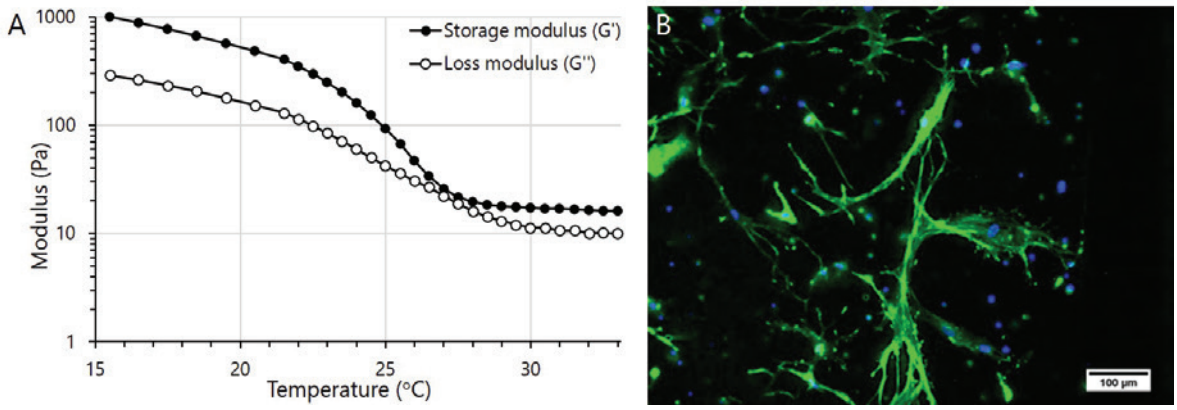


Figure 22. A) Storage and loss moduli dependence on temperature for GelXA BONE. B) Fluorescence microscopy image of osteoblasts after 35 days of culturing in ionically crosslinked GelXA BONE constructs. Cell nuclei: Blue. Actin filaments: Green. Scale bar: 100 µm.

GelXA SKIN

Description

GelXA SKIN is designed for fabricating skin and skin-related tissue models. GelXA SKIN crosslinks with thrombin, calcium chloride and through photocuring. GelXA SKIN incorporates well-dispersed fibrinogen to provide an environment resembling the native wound-healing process. Fibrinogen is converted to fibrin in the presence of thrombin, forming stable networks and promoting tissue repair. To fine-tune mechanical properties, users can photocure constructs after adding a thrombin-containing crosslinking agent.

Research application

We used GelXA SKIN to generate skin models and co-cultures of human dermal fibroblasts and human keratinocytes, incorporating multiple layers to mimic natural skin. The bioink supported survival and proliferation of fibroblasts and keratinocytes as seen in the microscopy images of live and dead stained constructs (Figure 23A-B). The GelXA SKIN constructs also demonstrated production of collagen type I (Figure 23C) and keratinocyte maturation (Figure 23C). The skin tissue models produced with GelXA SKIN can be used to screen compounds and evaluate their responses to wound healing, regeneration and more.

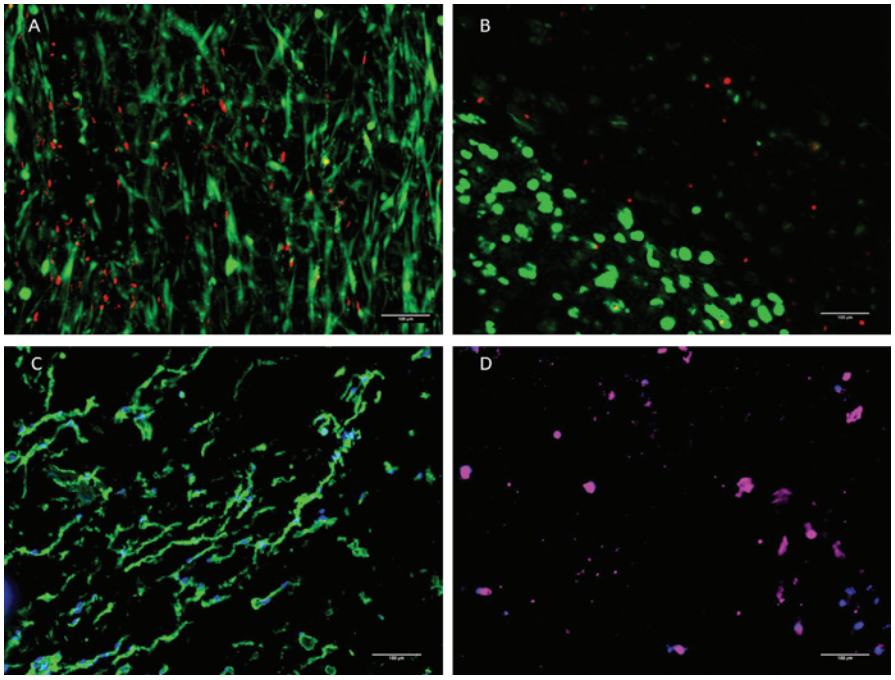


Figure 23. Bioprinted skin tissue models with GelXA SKIN laden with (A) fibroblasts and (B) keratinocytes with live (green) and dead (red) cell staining at day 14 of culture. Immunostaining of bioprinted skin constructs after 14 days to evaluate production of (C) collagen type I (green, nuclei blue) and (D) keratinocyte maturation keratin 10 (pink, nuclei blue). Scale bars: 100 µm.

GelXA FIBRIN

Description

GelXA FIBRIN incorporates fibrinogen to provide an environment that resembles the native wound-healing process. Fibrinogen is converted to fibrin in the presence of thrombin, forming a stable network and promoting tissue repair. Users can use photocuring to tune the stiffness of the bioprinted construct.

Research application

By converting fibrinogen into fibrin, GelXA FIBRIN can aid wound-healing studies with enhanced fibroblast activity, which is demonstrated in our in-house model (Figure 24). At day 14 of culture, the fibroblasts proliferate and migrate throughout the construct, forming a cellular-based network. These models can be used to evaluate the wound-healing capacity of the fibroblasts with and without compound enhancements.

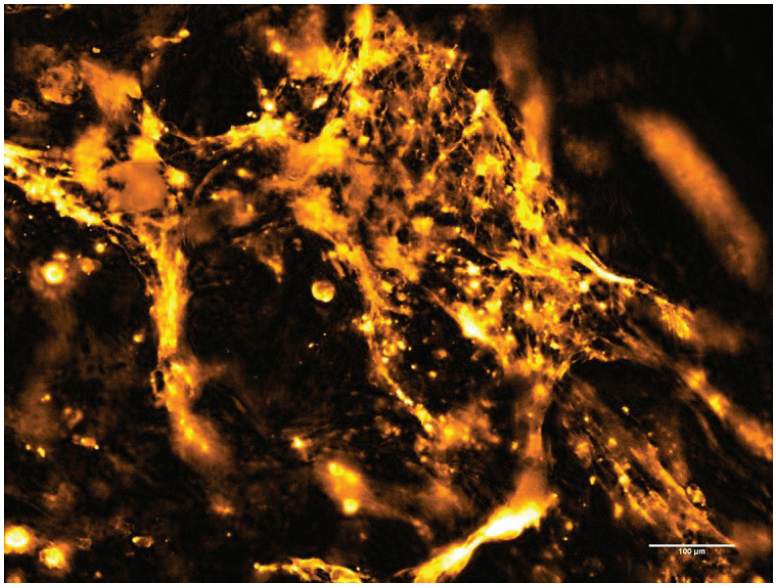


Figure 24. Human dermal fibroblasts bioprinted in GelXA FIBRIN as a wound-healing model stained for actin (orange) at day 14. Scale bar: 100 µm.

GelXA LAMININK

Description

The GelXA LAMININKs include GelXA LAMININK 111, 121, 411, 521 and +. These bioinks incorporate different laminin isoforms to mimic the basal lamina of the natural tissue environment. Laminins are essential to cell differentiation, migration and adhesion, and help support a healthy tissue. The GelXA LAMININKs are designed to enhance tissue maintenance and survival. These bioinks maximize cell viability and are a great starting point for culture of many cell types. Compared to other heterogenous matrices, our laminins' xenographic source enables better control over the matrix environment.

Research application

The diverse selection of GelXA LAMININKs enables researchers to bioprint all cell types (animal, human, progenitor and stem cells) and generate complex tissue models. These tissue models can be used to investigate tissue regeneration, cell differentiation/maturation, rejuvenation, drug response and more. We conducted a drug response experiment on liver tissue models, using GelXA LAMININK 111 for the co-culture of HEPG2 and LX2 cells (Figure 25). The bioprinted liver tissue constructs were dosed at day zero with different concentrations of a toxic reagent to determine cytoxicity of the cells over a period of seven days. Even though the cells demonstrated high cytoxicity levels after two days, they were able to recover from the introduction of the reagent after five days.

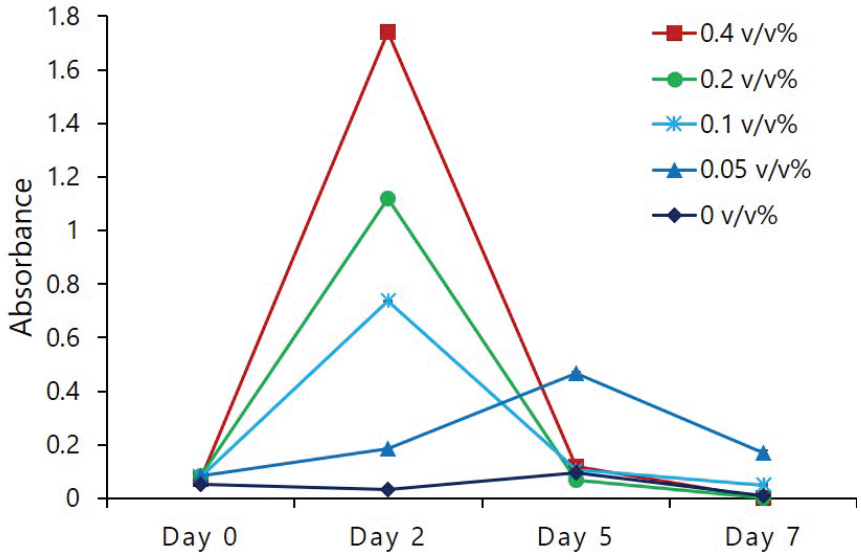
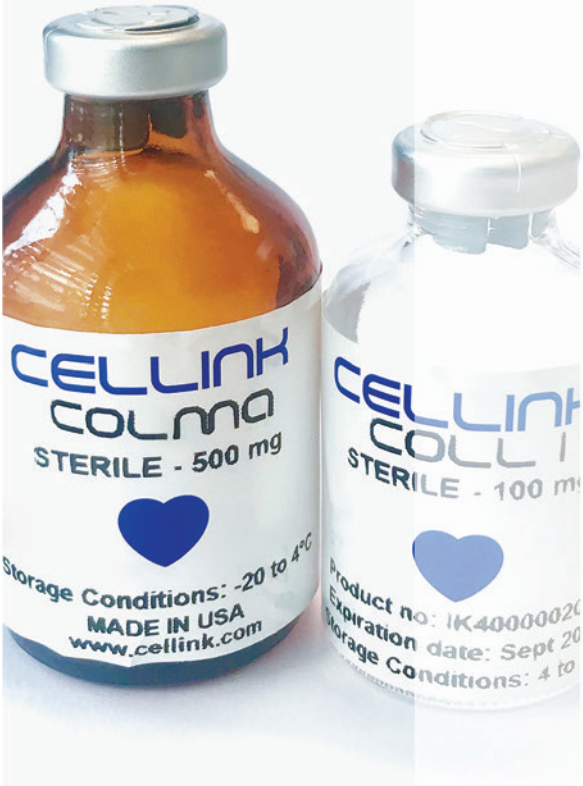


Figure 25. Dosing of toxic reagent on liver tissue models bioprinted with GelXA LAMININK 111.



COLLAGEN SERIES STORY

THE BIOINK WITH NATURE INSIDE

Type I collagen is one of the main structural proteins found in extracellular matrix (ECM) and connective tissue. Collagen is found in over 30% of total body proteins, making it an excellent biomaterial for tissue engineering and 3D printing applications. Collagen forms fibrous networks in the body, enhancing the structural integrity of the ECM while promoting cell adhesion, growth, biological signaling and tissue morphogenesis.

We developed the Collagen series using type I collagen (Coll 1) and collagen methacrylate (ColMA) variations intended for applications in 3D cell culture, surface coating for regenerative medicine and many more. CELLINK aims to leverage collagen's unique native physiological properties in an innovative formulation that propels research further. We source collagen from high-quality rat tail tendons to offer pure and sterile Coll 1 and ColMA.

We offer collagen as a reconstituted solution and freeze-dried powder, enabling you to customize your biomaterial formulations and create printable hydrogels, supplemented cell culture coatings and 3D cell culture matrices.

To learn more about the Collagen Series or get a quote, visit www.cellink.com/bioinks/collagen-series. You can also the QR code with your smartphone to go straight to our website.

UNIQUE BIOINK CHARACTERISTICS

We pay great attention to the rheological properties and crosslinking capability of our collagen-based biomaterials because they are integral to the success of your research. You can use the CELLINK Collagen series in multiple ways. Coll 1 can be printed as a 3D cell culture model between 15 and 25 degrees Celsius, and thermally gelated at 37 degrees (Figure 26). Rapid gelation occurs within a few minutes with the highest storage modulus increase for the highest concentration of Coll 1 (Figure 27A). ColMA is a unique variation of type I collagen that has been modified with photoactive methacrylate groups, enabling it to be crosslinked with 365- and 405-nm wavelength light. Crosslinking makes it possible to obtain robust constructs with well-controlled mechanical properties that mimic the physical properties of native tissues (Figure 27B). You can mechanically extrude collagen formulations with higher viscosities for added control over the forces endured by cells.

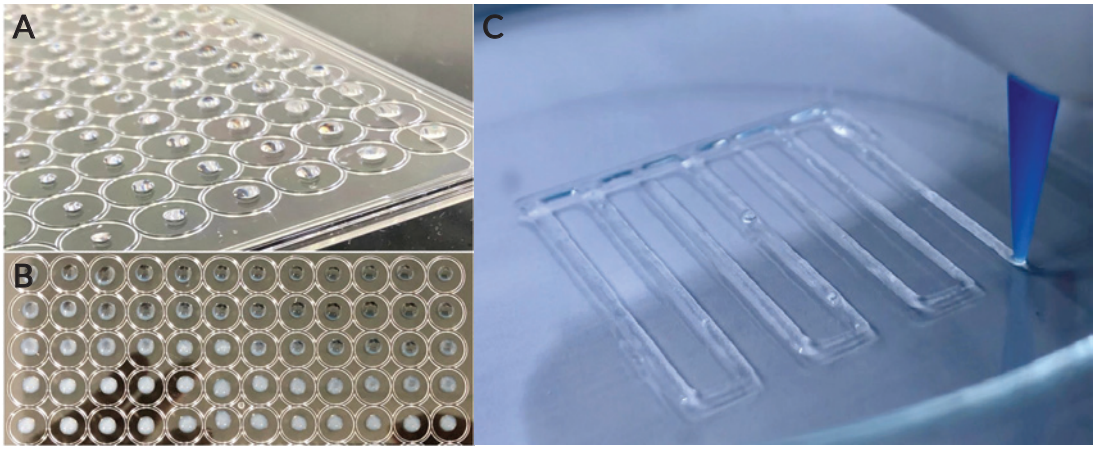


Figure 26. Droplets printed with Coll 1 biomaterial (5 mg/mL concentration) A) before and B) after different stages of thermal polymerization. C) 3D-printed ColMA filaments (10 mg/mL concentration).

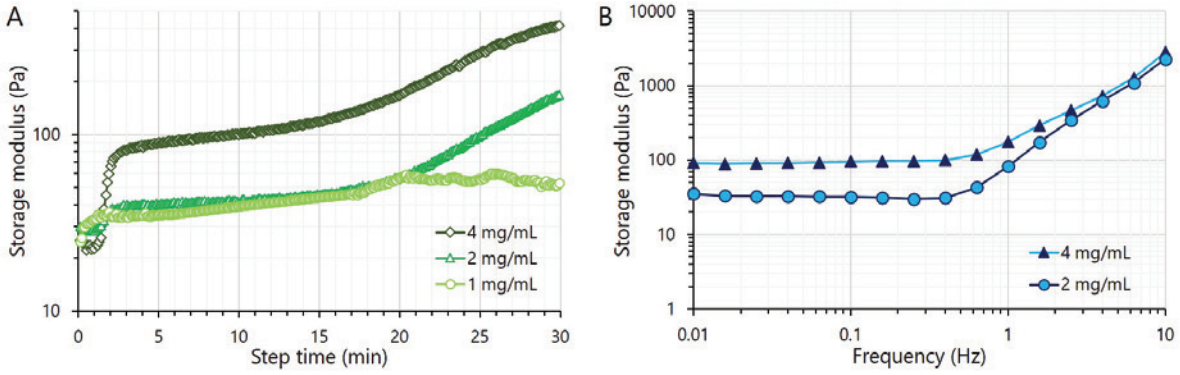


Figure 27. A) Storage modulus increase for Coll 1 at 37 degrees as a function of time. B) Storage modulus dependence on shear frequency for photocrosslinked ColMA at 2 and 4 mg/mL concentration.

Coll 1

Coll 1 supports cell proliferation on both 2D and 3D levels

Type I collagen is a well-known coating biomaterial used to enhance cell adhesion and proliferation. After seeding on top of our Coll 1 biomaterial, human chondrocytes from the femoral head demonstrated excellent viability (Figure 28, light green bars), cell proliferation and stretching (Figure 29, top row). However, embedding the living cells into a soft biomaterial like collagen is a much more challenging task to accomplish. We observed successful proliferation of human dermal fibroblasts embedded into thermally gelated Coll 1 biomaterial. After the initial drop in cell viability related to the 3D printing stress, the cells show great recovery by day seven and very good cell viability at day 14 (Figure 28, dark green bars). The chondrocytes are homogeneously distributed inside the 3D-printed droplets, forming an extensive interconnected network (Figure 29, bottom row).

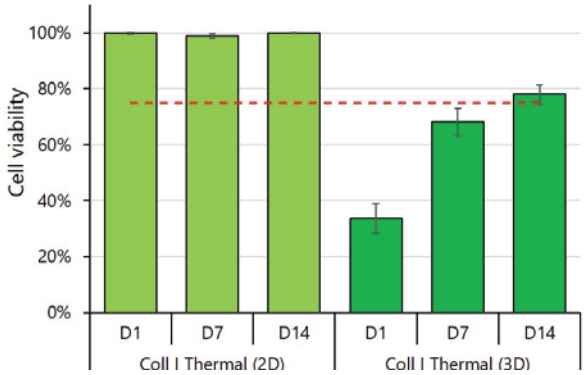


Figure 28. Cell viability analysis in the collagen-based constructs after one, seven and 14 days of culturing. Human chondrocytes derived from the femoral head were post-seeded on top of Coll 1 droplets (2D), and human dermal fibroblasts (HDFs) were embedded into Coll 1 droplets (3D). Cell viability is expressed as a percentage of live cells to the total count. The dashed line represents 75% cell viability threshold.

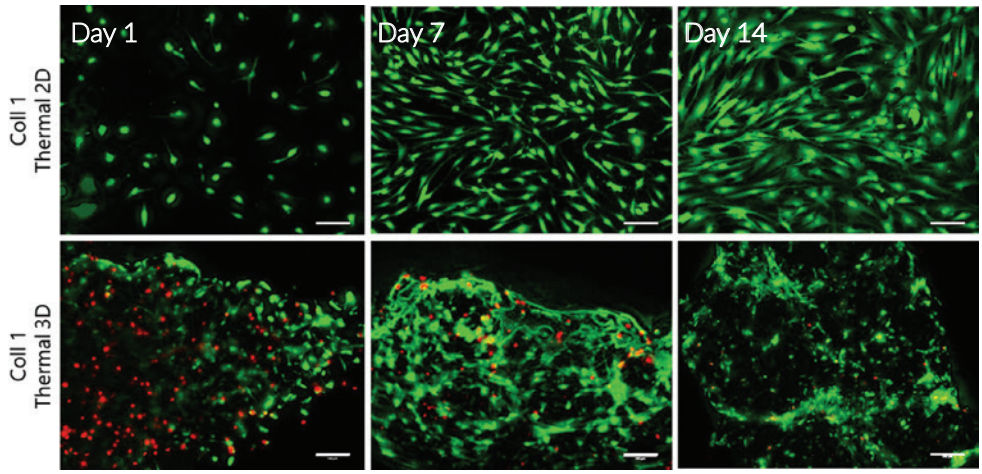


Figure 28. Top row: Fluorescence microscopy images of human chondrocytes (from the femoral head) after one, seven and 14 days of culturing on top of thermally crosslinked Coll 1 droplets. Bottom row: Fluorescence microscopy images of human dermal fibroblasts (HDFs) after one, seven and 14 days of culturing inside thermally gelated Coll 1 droplets. Scale bars: 100 µm.

ColMA

ColMA forms stable scaffolds after both thermal crosslinking and photocrosslinking

Human chondrocytes derived from the femoral head were post-seeded on top of ColMA constructs crosslinked with two different methods: thermally at 37 degrees Celsius and through methacrylic group photopolymerization using LAP photoinitiator and exposure to 405-nm wavelength light. Both methods were successful as chondrocytes showed excellent cell viability (Figure 30) and homogeneous cell distribution, especially after seven days of cell culturing (Figure 31). There was a rather minor drop in cell viability for photocrosslinked constructs, which can be attributed to the effect of near-UV light. Generally, photocrosslinking is the preferred method for forming ColMA constructs with embedded living cells.

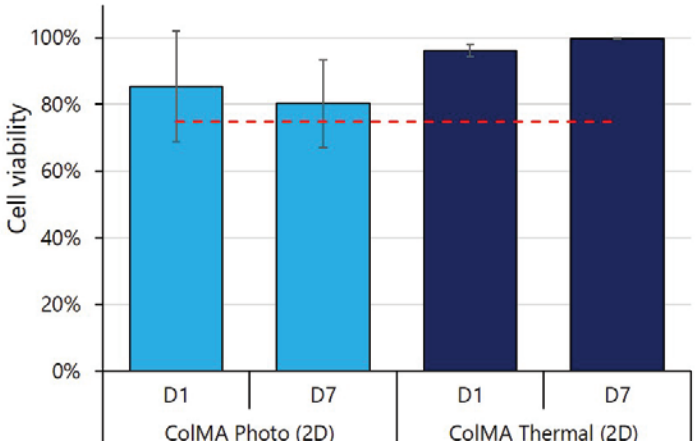


Figure 30. Cell viability analysis of human chondrocytes from the femoral head after one and seven days of culturing on top of the ColMA constructs. Cell viability is expressed as a percentage of live cells to the total count. The dashed line represents 75% cell viability threshold.

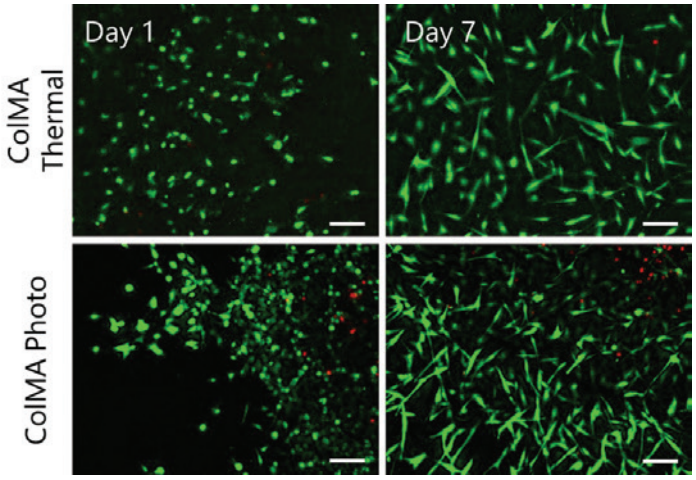


Figure 31. Fluorescence microscopy images of human chondrocytes from femoral head after one and seven days of culturing on top of the thermally crosslinked (top row) and photocrosslinked (bottom row) ColMA constructs.

CELLINK A SERIES STORY

SIMPLE AND POWERFUL

The A Series bioinks are composed of high-quality raw alginate materials to enable high reproducibility in your research. Alginate is an FDA-approved, naturally derived polysaccharide isolated from the cell walls of brown algae. Due to its versatility, it is used in many applications including drug delivery studies.

Alginate's biocompatibility and ease-of-use make it a useful material in tissue engineering applications, including bone and cartilage. Alginate can be modified to contain nearly any peptide sequence, permitting the fabrication of cell-specific biomaterials to guide cell differentiation and tissue formation. Our CELLINK A-RGD bioink is designed to leverage this property.

To learn more about the CELLINK A series or get a quote, visit www.cellink.com/bioinks/a-series. You can also scan the QR code with your smartphone to go straight to our website.

UNIQUE BIOINK CHARACTERISTICS

Alginate dissolved in mannitol solution ensures an excellent environment with the correct osmotic pressure for mammalian cells. Due to its translucent appearance, alginate minimizes optical interference when making both brightfield and fluorescent images. This enables imaging of several layers of cells in a 3D construct (Figure 32).

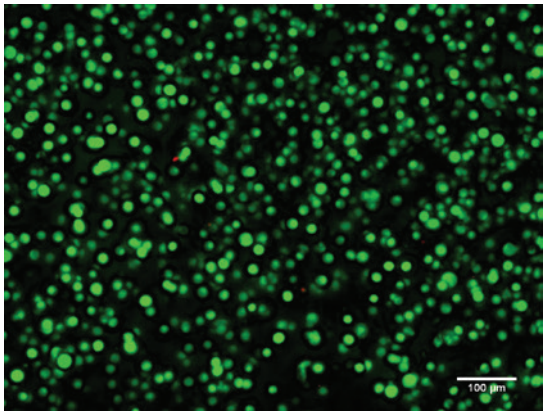


Figure 32. Live/dead staining of mesenchymal stem cells 3D cultured in CELLINK A for 14 days. Scale bar: 100 µm. (Live cells are green and dead cells are red.)

Divalent cations like the Ca^{2+} in our Crosslinking Agent bind adjacent chains in the alginate together to rapidly form a crosslinked gel. Prior to crosslinking, the bioinks are soft, viscous gels that are shear thinning at high shear rates (Figure 33A). The bioink becomes stiffer once crosslinked, making it easy to handle your bioprinted constructs during cell culture and imaging (Figure 33B).

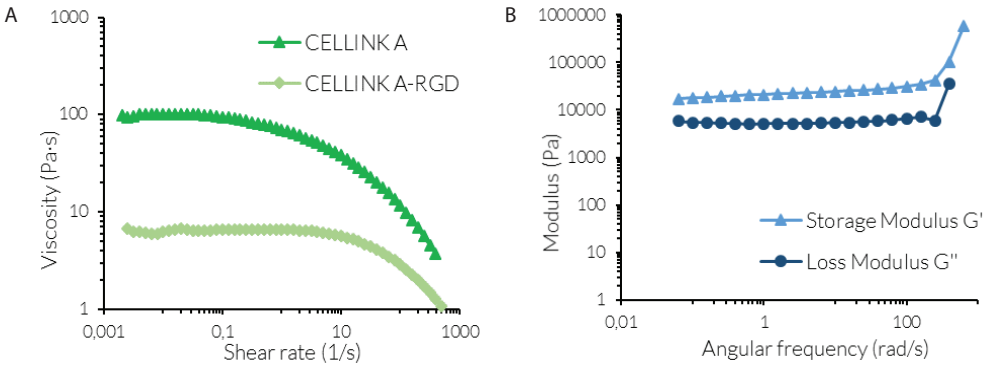


Figure 33. A) Viscosity behavior of bioinks over a wide range of shear rates. B) Loss modulus and storage modulus of CELLINK A-RGD crosslinked with Crosslinking Agent.

CELLINK® A

Description

CELLINK A is a viscous alginate hydrogel suitable for both bioprinting and casting. Its simplicity is also its strength — you can use it on its own, mix it with another hydrogel to make the blend crosslinkable with calcium ions or prepare an encapsulation of a cell-laden bioink. These are just a few potential applications — let your imagination run free!

Research application

One of CELLINK A's applications is histology sample preparation. Users can embed fragile and small constructs like organoids to enable easy downstream histology processes. CELLINK A is an excellent embedding material because it does not contribute to background staining. We used CELLINK A when preparing organoids for histochemistry (Figure 34).

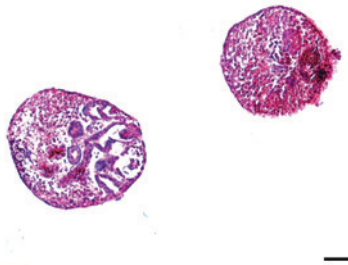


Figure 34. Organoids embedded in CELLINK A. Cells stained red and purple with trichrome staining. Scale bar: 100 µm.

One way of encapsulating a bioink with CELLINK A is using a technique called "drop-on-drop" bioprinting. A droplet of cell-laden bioink is printed (Figure 35A), and a larger droplet of CELLINK A is printed on top (Figure 35B). Crosslinking CELLINK A forms a protective shell around the cell-laden bioink to prevent it from dissolving. In addition, CELLINK A's translucence enables easy imaging of the encapsulated core.

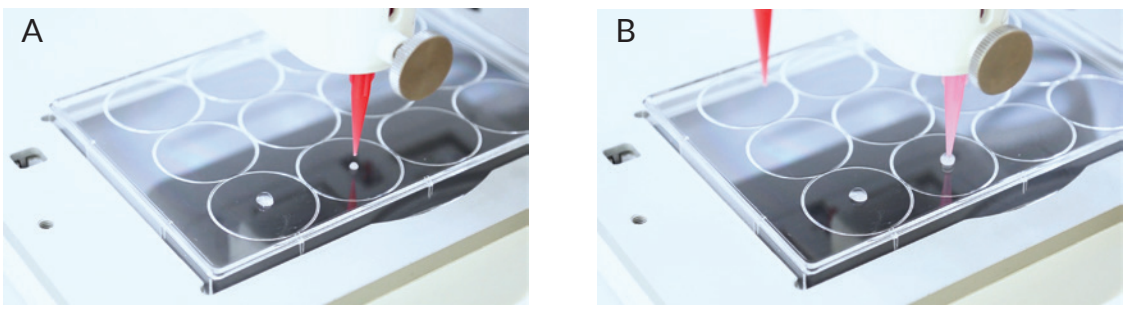


Figure 35. A) A small droplet of a cell-laden bioink is printed. B) A larger droplet of CELLINK A is printed on top of the first droplet, covering it.

CELLINK® A-RGD

Description

CELLINK A-RGD consists of alginate covalently conjugated with the L-Arginine-Glycine-L-Aspartic Acid peptide sequence covalently conjugated, also known as RGD. RGD improves cell attachment and has wide applicability in engineering nearly every tissue. The sequence has also been used for surfacing implants including bone and vascular implants to improve integration within the body.

Research application

Since CELLINK A-RGD enhances cell attachment. It can be used both on its own and as an additive in other bioinks. Simply add it to your desired bioink, mix in cells and start bioprinting — easy as that! To demonstrate the great cell attachment property, we seeded mesenchymal stem cells on top of crosslinked CELLINK A-RGD and cultured for 14 days (Figure 36).

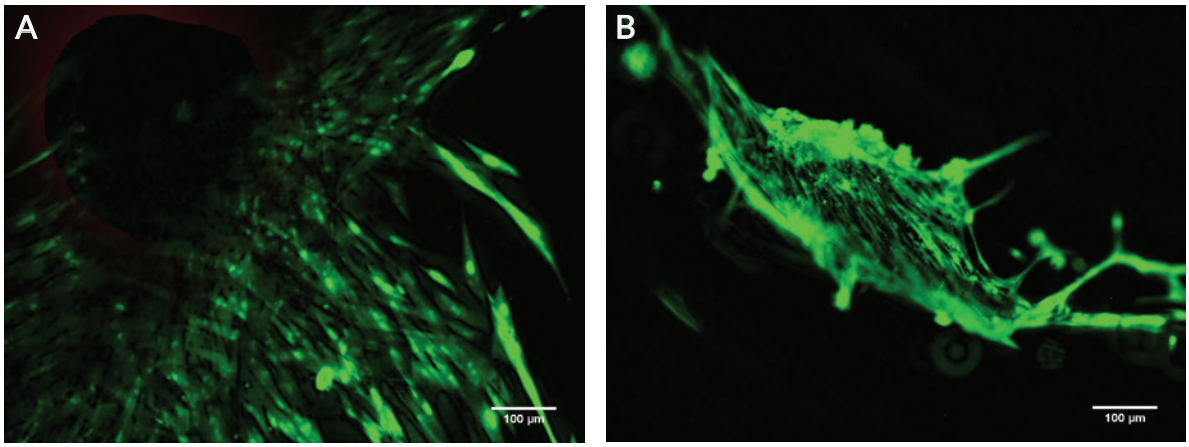


Figure 36. Live mesenchymal stem cells (green) seeded on top of CELLINK A-RGD at A) day seven and B) day 14. Scale bar: 100 µm.

With our bioprinters and bioinks, we are pioneering the next generation of medical research. We work together with our collaborators in hundreds of labs across more than 50 countries to deliver the best quality and support. The magnitude of the CELLINK team's knowledge, compassion and drive is enabling us to change the future of medicine.

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