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CELLINK®

CELLINK® LAMININ SERIES

The future of medicine is here

What is Bioink?

A bioink is a biomaterial that is suitable for bioprinting with cells and provides a temporary or permanent support to the cells while they produce their own extracellular matrix. Bioinks based on biopolymers, such as collagen, gelatin, hyaluronan, silk, alginate, and nanocellulose, are known for their favorable biocompatible properties and are attractive biomaterials for cell encapsulation and 3D bioprinting. These bioinks provide an aqueous 3D environment consisting of biologically relevant structural, physical, and chemical signals. Significant advances in 3D bioprinting technology as well as development of new bioinks have made it possible to bioprint complex 3D tissue structures.

Why Bioprinting? The innovative methods for engineering human tissues and organs can have a profound effect on the future of medicine. 3D Bioprinting is considered a revolutionary technology for advancing and accelerating progress in the field of tissue engineering and regenerative medicine, and thus, the future of medicine. We believe that we can create this future through a collaborative spirit and by putting our combined expertise to the service of humanity.

The future is created in the present and it belongs to the doers, those who continue moving forward in order to see their vision come to realization. It's not that we see the future and then move towards it. We move in order to see it.

WE ARE CELLINK

We are a team of entrepreneurs, scientists, engineers and pioneers, pushing the limits for what's possible, paving the way for the future of regenerative medicine.

With our 3D bioprinters and bioinks, we will open the possibility for more extensive medical research. Together with our collaborators, in hundreds of labs in over 45 countries, we work side by side to ensure quality and support.

Our compassion for humans and drive to move an impact will pave the way for continued growth.

"Thanks CELLINK for engaging the students and holding this successful workshop on bioprinting"- Ric Levato, UMC Utrecht

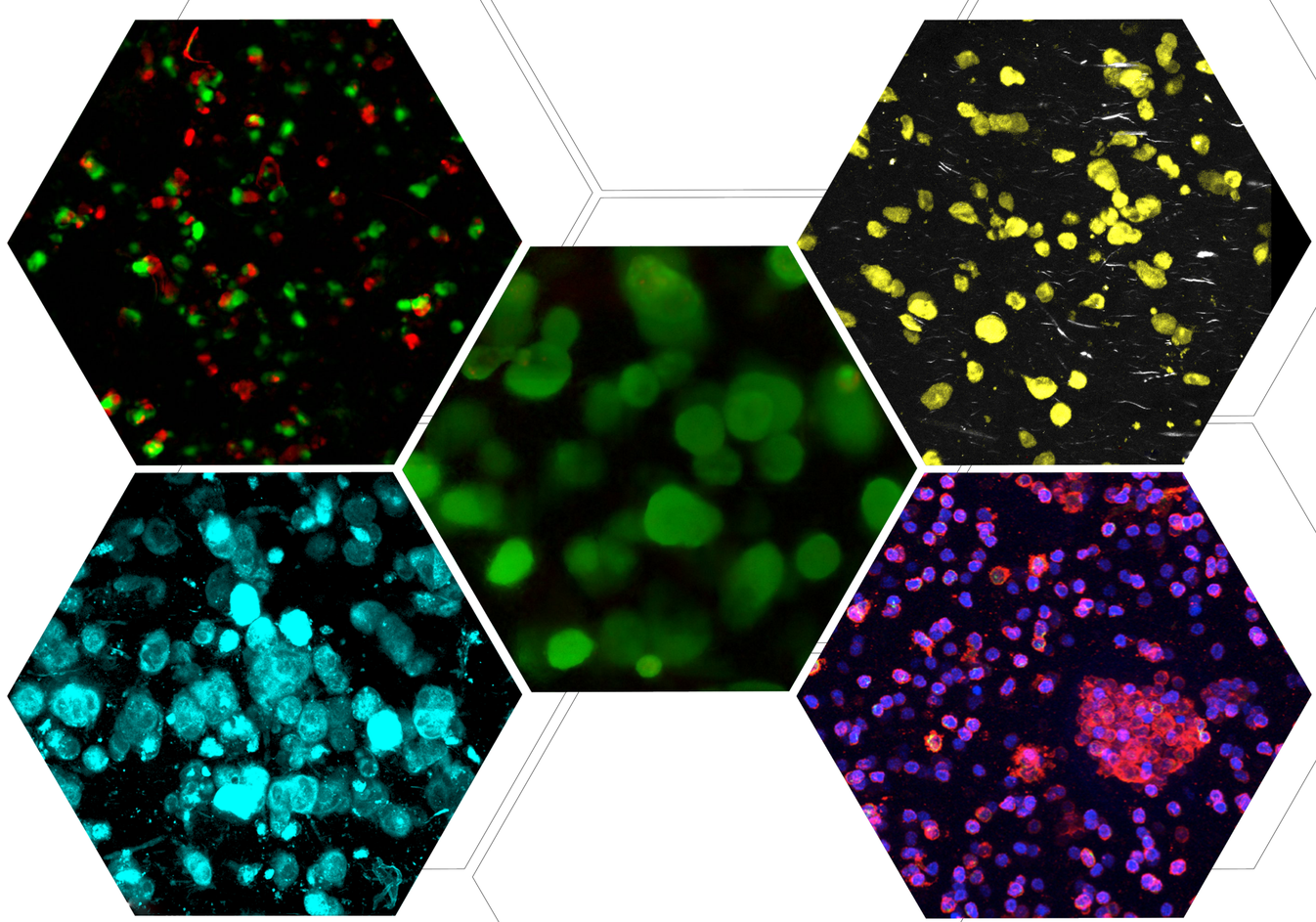
"CELLINK has taken our feedback and adapted their system while being actively engaged in the process" - Dr. Grande, The Feinstein Institute for Medical Research

CELLINK LAMININ SERIES

Laminins are proteins composed of three subunits referred to as a α-chain, β-chain and γ-chain. They are a major component of the basal lamina that lines the external surface of cell membranes. This protein is characterized by its cross-like structure that serves as a structural foundation for many tissues. Laminins play an important role in cell differentiation, migration, adhesion and also involved in supporting healthy tissue. Several laminins have been identified, which all have been related to different tissues and organs in the human body.

Our CELLINK LAMININ SERIES consists of five tissue-specific bioinks that have been developed for improving maintenance and survival of tissue. The bioinks contain laminins to mimic the basal lamina and the natural tissue environment. These bioinks retain the excellent printability and ease of cross-linking found with the universal CELLINK® bioink.

- LAMININK 111
- LAMININK 121
- LAMININK 411
- LAMININK 521
- LAMININK+



CELLINK® LAMININK 111

LAMININK 111 has been developed as a bioink for application with cells that require the presence of the laminin 111 isoform. Laminin 111 consists of a chain conformation of $\alpha 1\beta 1\gamma 1$ and it plays a role in the activity of many cell types.

LAMININ 111 is important during early development of the lining of tissues and organs. Specialized applications that this bioink is suited for include the culture of brain, kidney, liver or intestinal cells. In disease modeling, this bioink is relevant for studying Parkinson's disease and cancer models.

In our example below, LX2 cells were successfully 3D bioprinted in LAMININK 111 (Figure 1). The cell displayed clustering and high viability over 3 weeks of cell culture.

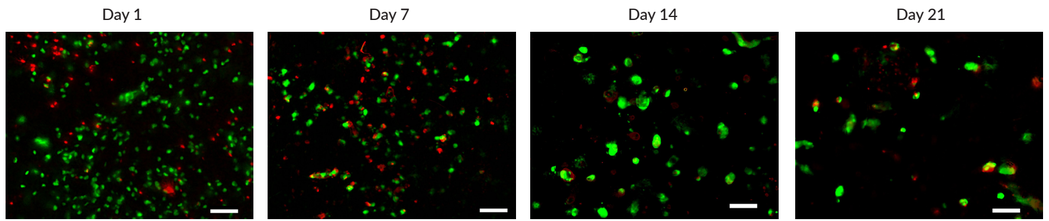


Fig 1. Viability images for LX2 in LAMININK 111 at 10 million cells/ml concentration. The green cells represent cells that are alive and the red represents dead cells. The cells were cultured up to 21 days. LIVE/DEAD staining. Scale bar 100 μm.

CELLINK® LAMININK 121

LAMININK 121 has been developed as a bioink for application with cells that require the presence of the laminin 121 isoform. Laminin 121 consists of a chain conformation of $\alpha 1\beta 2\gamma 1$ and it plays a critical role in the activity of many cell types. CELLINK® LAMININK 121 can be utilized with several cell types and this bioink can be utilized as a great starting point for the culture of many cell types.

LAMININ 121 is widely expressed during embryonic and early development of the lining of tissues and organs. This laminin variation is also found predominantly in the placenta, skeletal muscle, and kidney tissue. LAMININK 121 is particularly suitable as a base material for the culture of several cell types, including skeletal muscle, brain, kidney and liver cells.

In our lab, we have demonstrated the usefulness of LAMININK 121 by 3D bioprinting liver tissue models using Hep G2 cells. Within 7 days after bioprinting, the cells show proliferation with high viability (Figure 2).

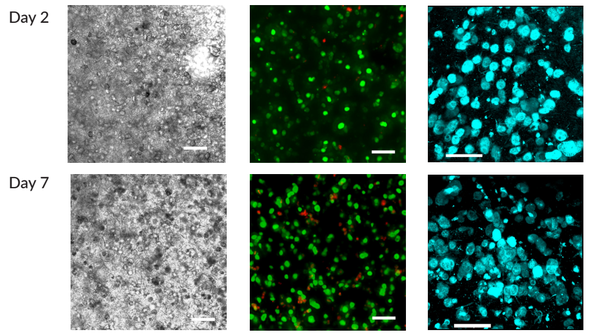


Fig 2. Indicated for day 2 and 7, to the left, brightfield images of cells evenly distributed within the LAMININK 121 cell-laden. Center, Live/dead staining images show minimal dead cells (red). Right, label-free microscopy images of Hep G2 (cyan, multiphoton microscopy). Scale bar 100 μm.

CELLINK® LAMININK 411

LAMININK 411 has been developed as a bioink for application with cells that require the presence of the laminin 411 isoform. Laminin 411 consists of a chain conformation of $\alpha 4\beta 1\gamma 1$ and it plays a critical role in the activity of many cell types.

LAMININK 411 supports many different tissues types including cells from the pancreas, vascular, immune, nervous, hematopoietic systems. This bioink is suitable as a base material for the culture of several specific cell types, including pancreatic cells and neurons. The bioink may be utilized as a base material for the development of pancreas models and the study of brain and nerve tissue.

In the example below, we 3D bioprinted human dermal fibroblasts, humanlung cancer cells and neuralprogenitor cells in LAMININK 411 (Figure 3). The cells showed high viability (green) and very few dead cells (red).

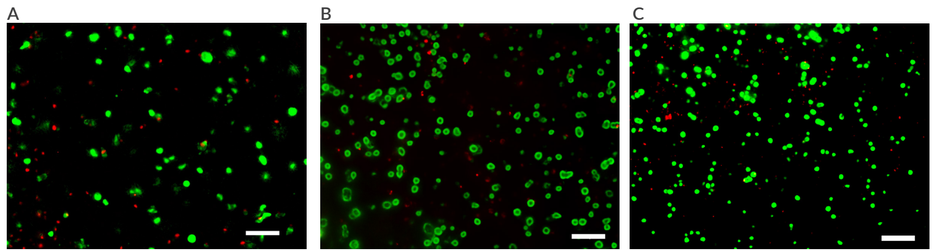


Fig 3. Viability images for: A. Human dermal fibroblasts in LAMININK 411 at 10 million cells/ml concentration. B. Human lung cancer cells in LAMININK 411 at 10 million cells/ml concentration. C. Human neural progenitor cells in LAMININK 411 at 10 million cells/ml concentration. The green cells represent cells that are alive and the red represents dead cells. LIVE/DEAD staining. Scale bar 100 µm.

CELLINK® LAMININK 521

LAMININK 521 has been developed as a bioink that supports cells that requiring laminin 521. Laminin 521 consists of a chain conformation of $\alpha 5\beta 2\gamma 1$ and it is important for the activity of many cell types. CELLINK® LAMININK 521 can be used as a great starting point for the culture of many cell types.
LAMININ 521 is the natural laminin for pluripotent and embryonic stem cells and therefore LAMININK 521 induces self-renewal of these cells. In addition, LAMININK 521 supports many diverse tissue cell types, such as cells from pancreas, vascular, nervous and muscular systems. Furthermore, the bioink can be used as a base for many specialized cells including hepatocytes, cardiomyocytes, and neurons.
The first example illustrates 3D bioprinted human dermal fibroblasts interacting with the LAMININK 521 matrix (Figure 4). The cells display proliferation, clustering and a healthy morphology. In the second example from our lab, human induce pluripotent stem cell derived cardiomyocytes clusters were 3D bioprinted in LAMININK 521 (Figure 5). On day one post-printing, cell clusters in the in the constructs were beating which demonstrates functional cardiac clusters. The cell-laden constructs were cultured for up to three weeks and showed predominantly healthy and viable clusters (green).

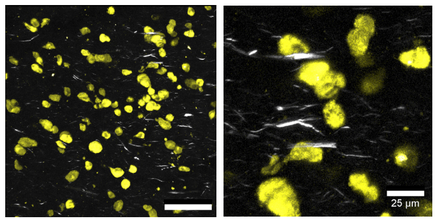


Fig 4. Microscopy images of 3D bioiprinted human dermal fibroblasts (yellow) in LAMININK 521 (white) (multiphoton microscopy). Scale bar 100 µm.

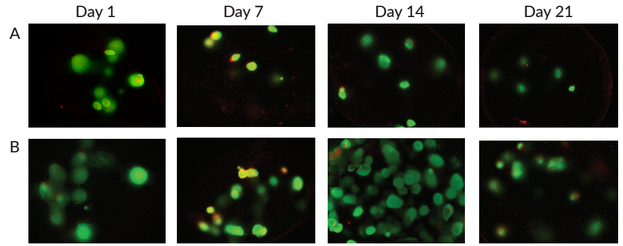


Fig 5. Viability images for clusters of human induced pluripotent stem cell derived cardiomyocytes in at A) 20 million cells/ml and B) 40 million cells/ml concentrations in LAMININK 521 cell-laden. LIVE/DEAD staining shows live cells in green and dead cells in red. 4X magnification.

CELLINK® LAMININK+

LAMININK+ has been developed as a bioink for application with cells that require the presence of many laminins. CELLINK® LAMININK+ is a blend of several types of laminins. This bioink retains the excellent printability and ease of cross-linking found with the universal CELLINK® bioink.

LAMININK+ can be used as a great starting point for the culture of many cell types. Our revolutionary blend of laminins within this bioink has been optimized to maximize cell viability.

In the example below, human dermal fibroblasts were 3D bioprinted using the LAMININK+ bioink (Figure 6). The cells showed high viability (green) and very few dead cells (red) up to 21 days.

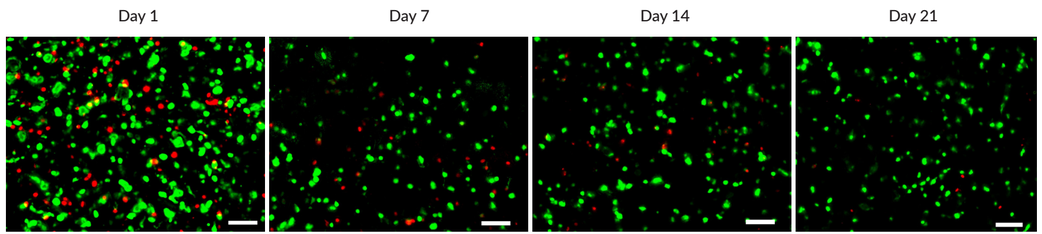


Fig 6. Viability images for human dermal fibroblasts in LAMININK+ at 10 million cells/ml concentration. The LIVE/DEAD staining shows a high viability (green). Scale bar 100 μm.

EASY PRINTING PROCESS

CELL MIXING

We have developed the easiest and most homogenous way of doing this using our innovative CELLMIXER. Put the bioink in the 3 mL syringe and your cells in suspension media in the 1 mL syringe. Clip each syringe to the dispensing unit, connect the mixing unit to the tip of each syringe and then connect the filling-cartridge. Fill the cartridge by gently injecting the ink and cells through the mixing unit. Your filling-cartridge is now ready for bioprinting and can now be disconnected from the mixing unit.

BIOPRINTING

When the cell mixing is done, and your cartridge is filled, you're ready to start printing. Screw a nozzle on to the cartridge and connect it to the air system. Now place it in the printhead. Continue by choosing the desired printing settings on the touch screen, such as temperatures, printing pressure, and printing speed. The parameters and the nozzle's diameter are chosen accordingly to the material of choice. Select the design you want and press print. BIO X will calibrate itself and start printing.

CROSSLINKING

Depending on the material you are printing, you may need to crosslink the printed construct. For UV crosslinking, you can turn on the built in LED and the BIO X will do all the work for you. For other types of crosslinking, you can add the crosslinking agent directly on your construct.

1 2 3

Professor Cristina Scielzo
San Raffaele Scientific Institute, Division of Experimental Oncology, Italy

The focus of my research is to study the ill-defined cross-talk between the leukemic cells and their microenvironment. To study in depth, the nature of this cross-talk, we aim to re-construct a more physio pathological-like in vitro model using the 3D bioprinting strategy.

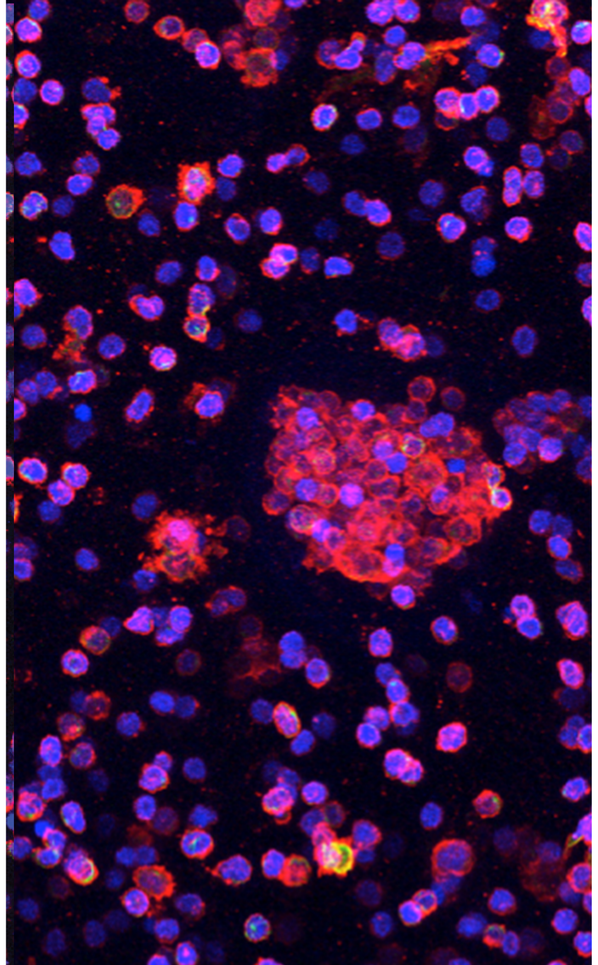
We are taking advantage of primary cells isolated from leukemia patients' or healthy donors' peripheral blood. We are mixing the cells with bioinks specifically designed to support cellular attachment and functions. The CELLINK LAMININ Series demonstrates high printability and biocompatibility.

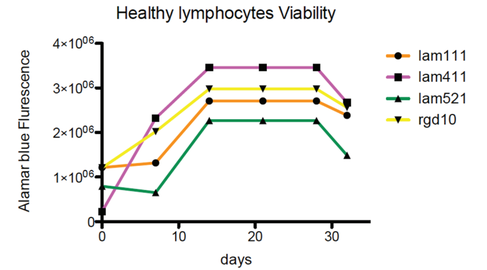
In particular, with the aim to recreate different stimuli derived from the extracellular matrix, we are testing in parallel different bioinks within the CELLINK® LAMININ Series and comparing to CELLINK® RGD10. We successfully tested leukemic cells for printability, behavioral change and viability. We optimized the printing strategy and set-up protocols for performing analysis for cells viability, flow cytometry, RNA extraction, protein extraction and confocal microscopy.

We found that leukemic cells have a long-term viability (up to 21 days) in the 3D bioprinted scaffolds (Figure 7).

Finally, using the CELLINK® LAMININ Series we found a different localization pattern of the cells, in particular more aggregates were observed with LAMININK 111 (Figure 8).

Our preliminary results show that we can efficiently 3D bioprint and follow leukemic cells over time in 3D allowing for more reliable physiological cell-cell interactions. Moving from a 2D culture system to more challenging 3D method, we will be able to study the behavior of leukemic cells in relationship to other cells and different extracellular matrix at basal level and when exposed to anti-leukemic compounds.





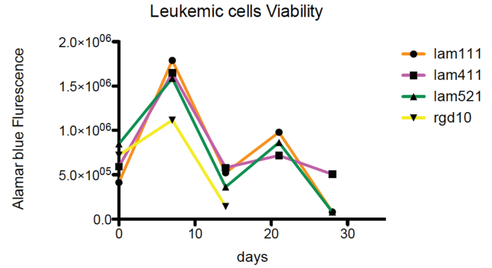


Fig 7. Alamar blue viability assay on representative healthy lymphocytes and leukemic cells at different time points and gels.

Fig 8. Confocal microscopy of one representative 3D bioprinted leukemic cells in Laminin111 gel after 14 days of culture. In red phalloidin staining and in blue Dapi.

Dr. Lisa Oliver & Dr. Celine Salaud
Institute of Health and Medical Research, University of Nantes, France

The brain tumor is a complex and dynamic system consisting of various biochemical and mechanical cues that act in conjunction with its microenvironment. As such, a multicellular tumor in vitro model would be a powerful tool to resemble tumor pathophysiology.

We have attempted to develop a 3D biosphere model using Glioblastoma Multiforme patient-derived cultures (PDC) that would incorporate glioma cells with cognate cancer-associated fibroblasts (CAFs) or tumor-activated fibroblasts (TAFs). The effect of CAFs and/or TAFs on the proliferation, cell-cell interactions and response to treatment of PDC is being evaluated. We are using this model to reconstruct a simplified tumor using 3D bioprinting and then treating the tumor with a modified Stupp protocol (protocol used in clinic) to determine the role of the microenvironment on tumorigenesis. These types of experiments are being performed using the CELLINK LAMININ series.

The new CELLINK LAMININ series is so easy to use. The bioink is more fluid, making the mixing with cells much easier and quicker, thus less stress on the cells. The results obtained after bioprinting were great: the cellular structures were present after only ONE week and the distribution of cells and cellular structures appeared more uniform. More importantly, the cellular structures present resembled closely those observed in vivo.

Our initial observations show the formation of large aggregates of cells called neurospheres, which expand over time until the size of these neurospheres become to large and there is an expulsion and release of internal cells resembling a exploding volcano. This phenomenon we call the grow (initial proliferation of cells) or go (migration of cells) effect.

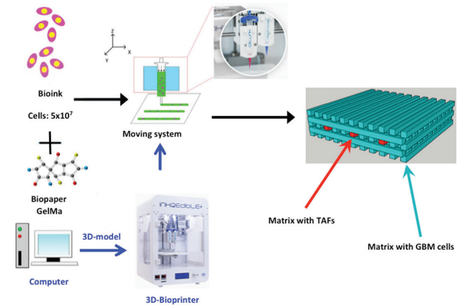


Fig 9. Illustration of the 3D bioprinting strategy.

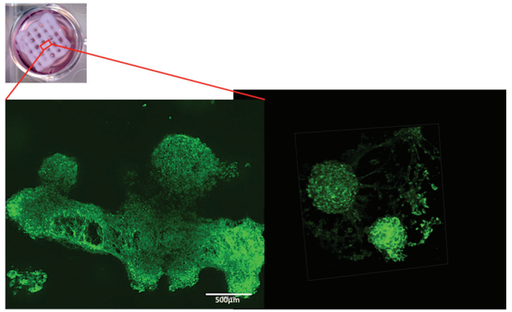


Fig 10. Organization of cellular network in the 3D bioprinted scaffold where the constructs were stained using actin (green).

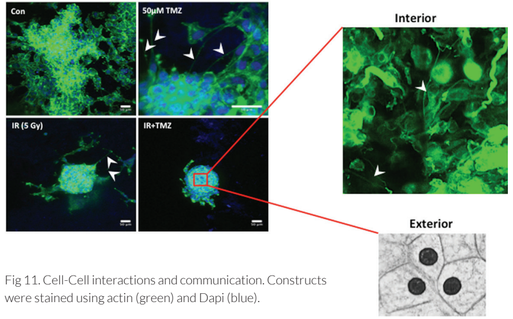


Fig 11. Cell-Cell interactions and communication. Constructs were stained using actin (green) and Dapi (blue).