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## Intercellular Mitochondrial Transfer Using 3D Bioprinting

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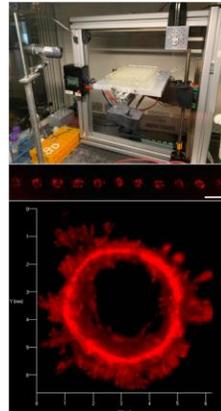
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## Abstract

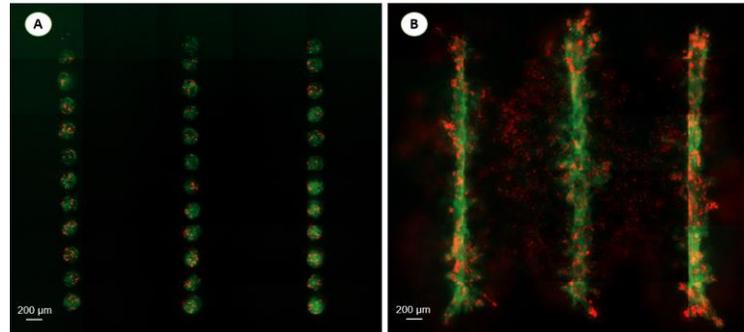
Mitochondria are one of the most complex and vital organelles in eukaryotic cells. In recent years, it has been shown that through intercellular mitochondrial transfer, this important organelle provides a critical role in tissue homeostasis, damaged tissue repair, and tumor progression under physiological conditions [1]. However, the mechanism of mitochondrial transfer and its effect on various cellular microenvironments has not yet been defined. Understanding the metabolic effects of mitochondrial transfer between cells and exploring the signaling leading to the intercellular mechanisms could provide advancements in both translational medicine and cell therapy for cancer progression and age-related diseases. Our group has studied the ability of the normal mammary microenvironment to redirect cancer cells to a normal mammary epithelial cell fate both *in vivo* and *in vitro* using our 3D bioprinting system [2-5]. Therefore, we sought to determine if mitochondrial transfer may play a role in mammary epithelium induced redirection of cancer cells. We used MCF-7 breast cancer cells and MCF-12a epithelial breast cells for experimentation. Using a fluorescent GFP-MITO lentivirus, we were able to mark mitochondrial protein in the MCF-12a epithelial cells to track mitochondrial transfer activity (figure 3). The MCF-7 cells were labeled red to distinguish the two cell types. The cells were then co-cultured in 2D tissue flasks and printed into hydrogels using the 3D bioprinter. Using fluorescent microscopy, mitochondrial protein was observed traveling from epithelial to mammary cancer cells. We hypothesize this is done for cancer cells to stabilize mitochondria and improve metabolic function and ATP production. Further research to establish mitochondrial transfer, its mechanism(s), and molecular effects could lead insight into how this cellular communication rescues and normalizes metabolic factors of the mammary and stem cell microenvironment leading to potential fate redirection and cellular revitalization.

## 3D Bioprinting

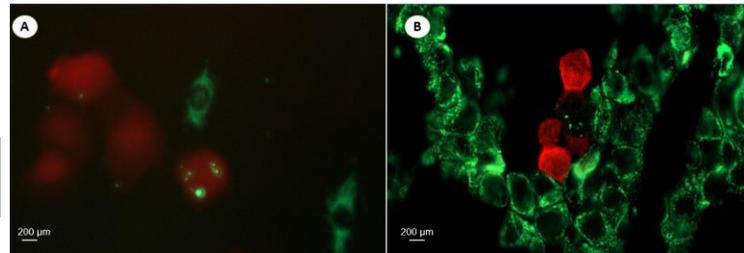
- Felix 3.0 fusion deposition method 3D printer from FELIXrobotics
- Low-cost open access 3D bioprinting system with high-throughput and precision
- Generates consistent and uniform cellular structures within various 3D hydrogel systems supporting stem cell and cancerous organoid growth
- A culturing mechanism for studying microenvironmental behavior
- Allows for mechanistic insight into cellular growth process and communication
- Through coding programming, the open-source printing platform can control cell placement within preformed hydrogels down to approximately 10µm in all three-dimensional planes of the X, Y, and Z [4]. This technological innovation makes it possible to accurately explore the presents of mitochondrial transfer in 3D structures and further examine the signaling required for microenvironmental control of fate determination.



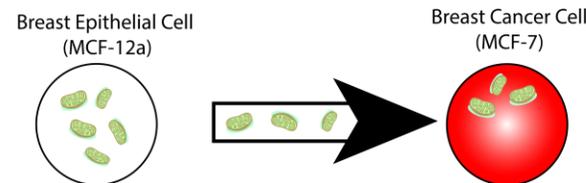
## Results



**Figure 1. 3D Printing Using Bioprinter.** A) Picture of 3D printed gel image. The image was taken on day 1 of printing and features RFP-tagged MCF-7 cells and MITO-GFP-tagged MCF-12a cells printed in rat-collagen gel in a three column, twelve row formation. The cells were printed in a 5:1 ratio of RFP to MITO-GFP and each well features approximately 100 cells per well. B) Image of 3D printed gel image on day 5 featuring same cells as in the previous image. Both images captured using Zeiss Zen fluorescent microscopy. Size standard for images is 200µm.



**Figure 2. 2D and 3D Evidence of Mitochondrial Transfer.** A) Co-culture of RFP-tagged MCF-7 cells and MITO-GFP-tagged MCF-12a cells in T75 flask in 12a culture media. The image was obtained 5 days after initial passage. The cells were passed in a 5:1 ratio of RFP to MITO-GFP. B) Image of fixed sectioned slide obtained from 3D printed gel image. Immunohistochemistry was performed on sections. The gels were fixed after 7 days and then sectioned. Both images captured using Zeiss Zen fluorescent microscopy. Size standard for images is 200µm.



**Figure 3. Mitochondrial transfer from MCF-12a to MCF-7 cells.** Mitochondrial protein from MCF-12a epithelial breast cells were labeled using a pCT-Mito-GFP lentivirus for mitochondrial tracking. MCF-7 breast cancer cells were transduced with a CAG-RFP lentivirus for cell type identification. The two cell types were co-cultured at 1:1 and 1:5 ratios in both 2D and 3D experiments. The green labeled mitochondrial travels from the MCF-12a epithelial cells to the red labeled MCF-7 cancerous cells.

## Conclusion

With the project proposed, we aim to gain a better understanding of microenvironmental communication through mitochondrial transfer and how this effects cellular homeostasis and potential cellular fate redirection. Using our novel 3D bioprinter, we can precisely and accurate study microenvironments in 3D hydrogels to better mimic *in vivo* experimentation. We demonstrated that MCF-12a epithelial breast cells labeled with a GFP-tagged mitochondrial vector (used to identify the transfer of the organelle within or between cell types) and MCF-7 RFP breast cancer cells successfully form and grow organoid structures in rat tail collagen hydrogel 3D cellular (figure 1). Furthermore, we showed that there is evidence of mitochondrial transfer from the donor MCF-12a cells to the recipient cancerous MCF-7 cells in both 2D and 3D co-cultures (figure 2).

## Future Research

Having established that mitochondrial transfer occurs between breast epithelial MCF-12a and cancerous cells types MCF-7 using 3D bioprinting, we hypothesize this microenvironmental communication as a way of explaining metabolic fate redirection and cellular rejuvenation. To continue this project, we aim to:

- Evaluate the frequency and to what extent the mitochondrial transfer is detectable
- Explore changing metabolic factors of cancer cells and stem cells after mitochondrial transfer
- Establish TNT as the primary mechanism for MT both in cancer and stem cell microenvironments

The work will be completed to better understand cell communication and mitochondrial trafficking on the molecular level to enhance advancements in therapeutic disease treatments and prevention

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