

## P17

# Cell cultured in 3D scaffolds analyzed by second harmonic images

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In this proposal we introduce a functionalized Hyaluronic Acid (HA) scaffold for 3D cell growth. The functionalization of the HA scaffolds was made using self-assembly peptides with laminin adhesion sequence. The resulting scaffold is named HA-EAbuK-IKVAV scaffold and it was used to seed cells of different cell lines. From preliminary results it shown Extra-Cellular-Matrix (ECM) with collagen fibers deposition [1]. The ECM at Masson trichrome histological staining showed collagen area depositions depending on the seeded cell type (e.g. breast cancer, melanoma, cervical cancer, etc.). Cells were seeded in the scaffolds after scaffold hydration with cell medium and cultured three days before analysis.

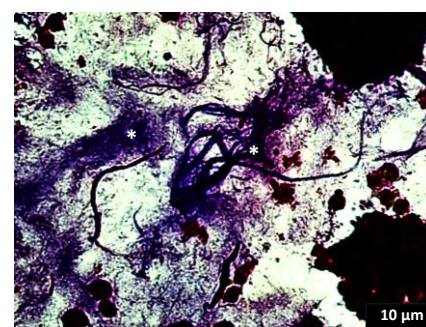
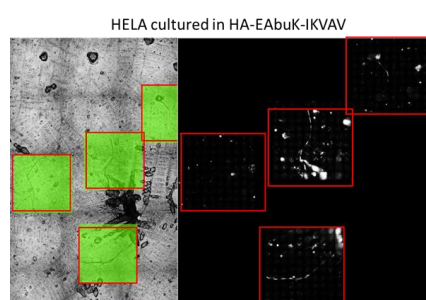


Fig. 1 Example of Masson trichrome staining. Blue area with white stars is collagen.



ig. 2 Example of second harmonic

After three days from the seeding the cultured scaffolds were verified for cell viability and collected for further analysis. They were positioned on a microscope slide with formalin for cell preservation and air dried for histological staining and analysis at biphoton. Fig. 1 shows an example of Masson trichrome staining of Hela cells cultured in HA-EAbuK-IKVAV scaffold where blue area represents the collagen.

Collagen fiber network was imaged using custom-built wide-field second-harmonic generation (SHG) microscopy system [2]. Exemplary tiled SHG image is presented in Figure 2 (right). Corresponding brightfield image is presented in Figure 2 (left).

## ACKNOWLEDGEMENT

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## REFERENCES

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- [2] Padrez, Y., et Al., (2024). Computerized Medical Imaging and Graphics, 117:102440