MECHANOBIOLOGY OF FIBROTIC PROGRESSION

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Introduction

While genetic mutations initiate breast cancer, tumor progression is mostly influenced by changes in the tissue mechanical microenvironment, primarily related to fibrosis. Fibrotic progression, characterized by dense collagen deposition, promotes tumor stiffening, modulates immune cell infiltration, and stabilizes newly formed vascular networks, ultimately driving metastases. Current in vitro models fail to replicate the complexity of fibrotic progression, while long-term intravital imaging in rodents is hindered by ethical and technical limitations. The embryonated avian egg chorioallantoic membrane (CAM), a unique alternative model, allows fir real-time imaging of fibrosis in an in vivo organism. In this work, we used a CAM model to recreate three different progression states of a foreign-body reaction, at a single timepoint and implantation site. We used label-free nonlinear microscopy to quantify in vivo matrix stiffness and vascularization within the fibrotic microenvironments, consisting of implanted microscaffolds, repopulated by the host cells and blood vessels (Fig1a).

Methods

We produced three sets of microscaffolds on the same glass coverslips (Ø=12mm), shaped as grids with a pore size of 10x10µm^2,30x30µm^2,or 50x50µm^2, 80µm in height, by using two-photon polymerization of SZ2080. We implanted this array in live chick embryos, and we conducted the CAM assay 7 days post-implantation, as previously described by our group [1]. Ex ovo inspection of the imaging window was performed using two-photon emission microscopy (TPEF) and second harmonic generation (SHG) imaging. Ex vivo assessments were carried out by sacrificing embryos at embryonic incubation day (EID) 14. The CAM was paraffin-sectioned and stained with hematoxylin and eosin, or labeled with nuclei tracker and observed by fluorescence microscopy.

Results

Microscopy analyses at the implantation sites in chick embryos (Fig.1b,c) revealed tissue infiltration inside and around the microscaffolds. Neo micro vascularization inside the scaffolds highlighted capillaries 10 times shorter, 1.4 times thinner from the inner to the outer part of the scaffold and 200 and 17 times shorter and thinner, respectively, compared to unimplanted samples. Moreover, each scaffold type generated a different vascularized condition (Fig.1d). In the 50x50µm^2 ones microcapillaries had a vascular density 2.9-fold greater compared to the ones found in the 30x30µm^2 samples, and 200-fold greater than in the 10x10µm^2. SHG revealed the presence of a collagen-I layer that was preferentially orientated and had a density significantly lower than in peri-implant areas, and greatly varying with scaffold type.

Discussions & Conclusion

Microscaffold implants successfully reproduced different levels of fibrotic progression, mimicking tumor-associated stroma by regulating collagen synthesis and revascularization. The differences in vascular density and collagen content, across scaffold types, replicated microenvironments with three increasing levels of fibrosis, likely driving breast cancer progression. This study validates the embryonated avian egg as a versatile platform for modeling fibrosis, allowing real-time, ethical, and cost-effective imaging compared to in vitro and mammalian models. By replicating multiple fibrotic stages simultaneously, our model provides a platform to gain insights on tumor stiffening mechanisms, and possible countermeasures to contrast drug resistance.

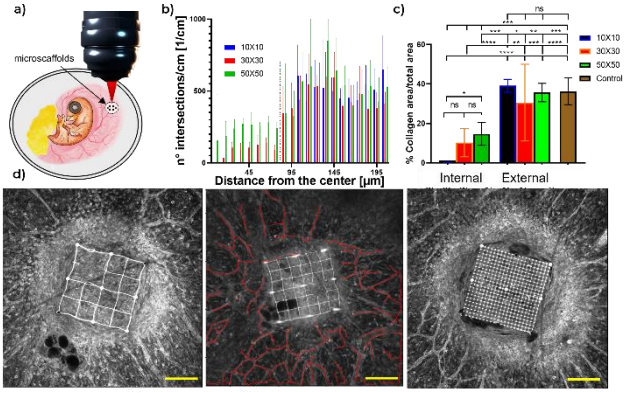

Figure 1: Panel (a): Sketched representation of the assay. Panel (b): blood vessels density counted in the microscaffolds and in the surrounding tissue as number of intersections per test line. Panel (c): percentage of inspected volume occupied by collagen fibers (SHG). In panels (d), we show the microscaffolds implanted in the embryo CAM taken under TPEF (λ_{Ex}= 910 nm, λ_{Em}:535/50 nm) at EID14 (scale bar = 100 µm). Fluorescence derives from cytoplasm and blood vessels.

References

1. Conci et al. 2023, DOI: 10.1063/5.0165411

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