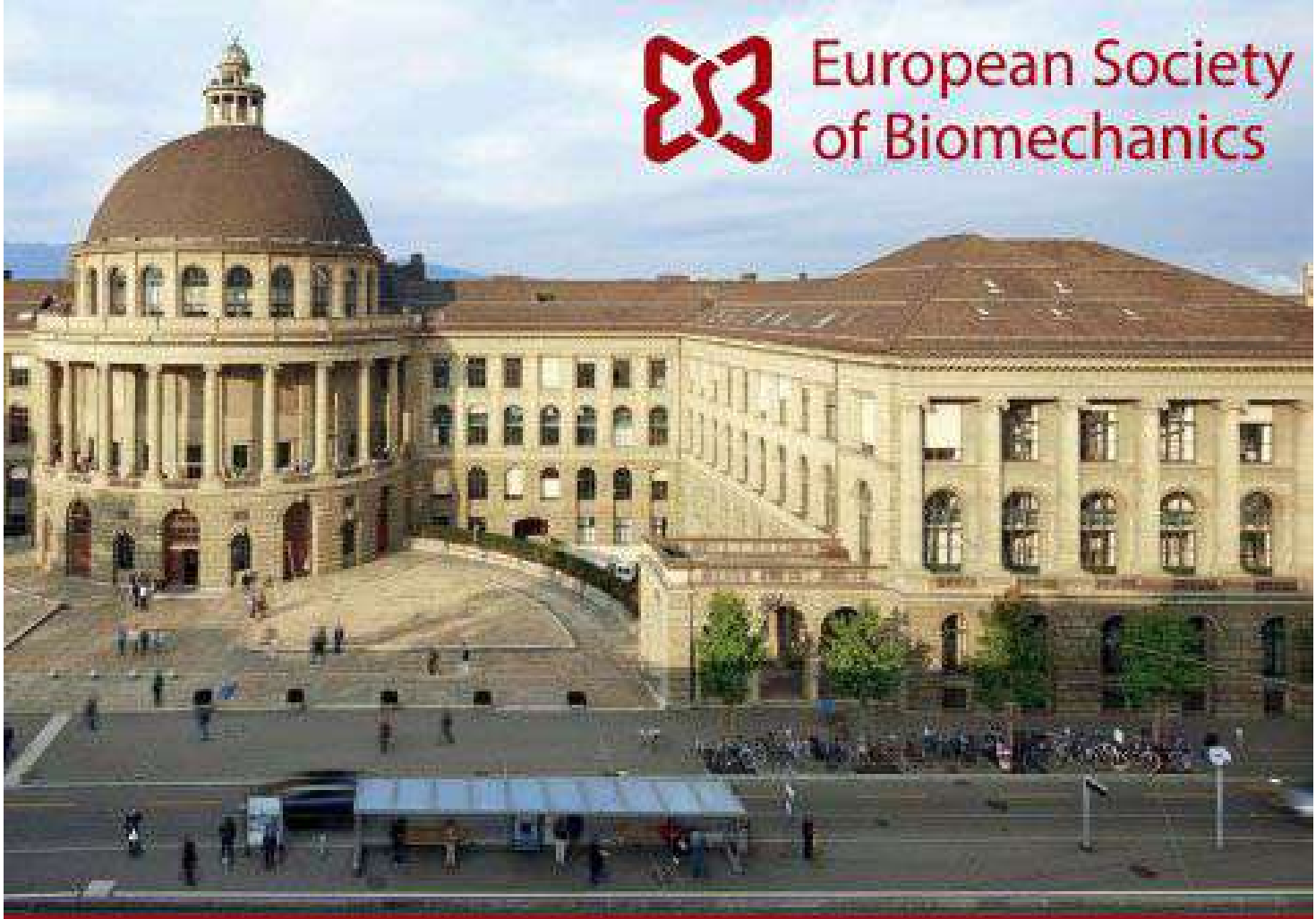

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CONTROLLING COLLAGEN POLARIZATION TO MODEL BREAST CANCER FIBROTIC PROGRESSION IN VIVO

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Introduction

Solid tumors progression correlates with extensive extracellular matrix (ECM) remodeling, characterized by progressive collagen deposition, cross-linking and fibrosis, stiffening the tumor microenvironment (TME) enhancing cancer cell proliferation and migration. In breast cancer, collagen fibers progressively align circumferentially around the growing tumor, forming a structural barrier that hinders drug delivery, accelerating tumor growth [1]. *In vitro* 3D models of growing tumors, such as spheroids and organoids, so-called tumoroids, recently emerged as a valuable tool for their ability to replicate aspects of the TME *in vitro* [2]. However, modelling fibrotic progression *in vitro* remains a major challenge due to the absence of key conditions present *in vivo*, such as functional vascularization. Our main goal is to use microscaffolds implanted *in vivo* to mimic different stages of fibrotic progression present in TME. In this study, we implanted newly designed scaffolds on the chorioallantoic membrane (CAM) of living chicken embryos, to exploit their foreign-body response (FBR) to recapitulate collagen orientation found in fibrotic progression.

Materials & Methods

We designed and fabricated two different scaffolds geometries on a 12-mm glass coverslip via two-photon polymerization (2PP), employing SZ2080, a biocompatible photoresist. While both architectures featured a central lattice structure measuring 250×250×80μm^3, with single pores of 50×50×20μm^3 (Fig.1 a-b), one configuration presented a surrounding array of free-stand pillars. This consists of three rows of 5×5×60μm^3 pillars inter-spaced 50μm (Fig.1a). We implanted these microscaffolds on the CAM and after 7 days, we dissected the implant region to analyze collagen I content via second harmonic generation (SHG). Scaffolds fluorescent contribution was manually excluded from images, before proceeding with image restoring algorithms. Collagen density was extrapolated on binarized images as the ratio of white pixels over the total pixels number. We analyzed orientation of collagen fibers surrounding the scaffolds, exploiting compass mask method. We employed 360 custom-designed edge-detection kernels (33×33 pixels, 11.2×11.2μm), strategically designed to detect edges aligned with collagen fibers at various orientations. Then, we quantified their alignment relative to the scaffold centre, by assigning an orientation score ranging from 0 to 1, corresponding to radial and circumferential alignment, respectively.

Results

SHG images analysis showed the capability of the two implanted configurations to elicit different FBR. The plain lattice induced a collagen infiltration (Fig.1c-right) in the inner pore portions equal to 9.33% and in the peri-scaffold region equal to 22.88%. Moreover, collagen fibers appeared randomly oriented, resulting in a disorganized architecture (Fig.1d-right). In contrast, the pillar array configuration (Fig.1c-left) while highlighting in the internal region a similar collagen content (7.92%), the external architecture (i.e. presence of pillars) produced a denser fibrotic capsule (54.50%) with a more aligned collagen content encircling the pillars in a circumferential pattern (Fig.1d-left).

Discussion & Conclusion

We used different architectures of microscaffolds implanted in a CAM model, to modulate a FBR by directing the resulting spatial organization of ECM fibers secreted by fibroblast cells that invaded the structures. The pillar array concept successfully mimicked the circumferential alignment of collagen fibers, characteristic of peritumoral fibrosis in breast cancer [1]. Our ground-breaking results highlighted the potential of mechanobiological signalling exerted by microscaffolds to finely modulate ECM remodelling to mimic cancer-associated matrix alterations.

Figures

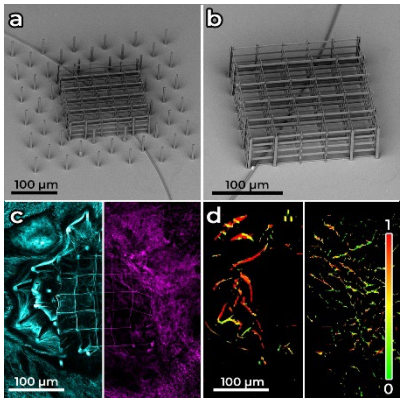

Figure 1: SEM images of the microscaffolds a) with and b) without the pillar array. c) SHG images of collagen fiber arrangement at day 7 post-implantation on the CAM, with (cyan) and w/o pillars (magenta). d) Color map of collagen fiber orientation, extracted from SHG images.

References

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2. Offedu et al., 10.1002/advs.202402757, 2024.

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