3D colonization of biomimetic tendinouscollagen scaffolds

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Tendons connect muscles to bones and transmit forces between them. They are mainly composed of type I collagen fibrils, aligned on average along the bone-muscle axis and arranged in a hierarchical fibrous structure. Collagen fibers fasciclesexhibit a periodic waviness, called “crimp”. This supramolecular structure provides tendonswith their unique mechanical properties.
Tendon injuries are very frequent and standard therapies have a low success rate. For this reason, *in vitro* tendon engineering represents animportant clinical challenge. Furthermore, the complex collagen arrangement in a tendon cannot be reconstituted without the action of specialized cells.
Weare currently developinga bottom-up approach based on the assembly of a biomimetic collagen scaffold which can be colonized by cells, first on the surface then in the bulk where they can eventuallyreorganize the matrix.

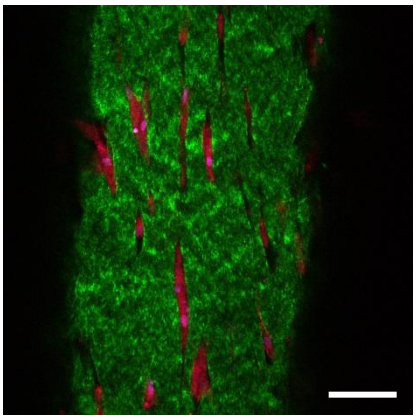

Figure 1: Second Harmonic Generation microscopy of a collagen thread at 60mg/mL, 30 µm under the surface, with C3H10T1/2 cells (green: fibrillar collagen; red: cell fluorescence), scale bar=50 µm.

Concentratedacidic collagen solutions (up to 60 mg/mL) are extruded in a neutralizing buffer which triggers their fibrillation.We thus obtain collagen threadsin a reproducible and standardized manner.Extrusion/fibrillationand the subsequent aging result in multiscale surface and bulk structures.
A characteristic surface roughness, akin to the “shark skin” pattern exhibited by polymer melts[1], is observed for the most concentrated solutions. This can be explained owing to their strong viscoelasticity.
More unexpectedly, Second Harmonic Generation microscopyreveals thatcollagen fibrils tend to alignat a sharp angle with the thread axis inside a shell of age-dependent thickness, while the core remains isotropic.

Finally, the collagen threadswere seeded with mesenchymal stem cells under tension. After two weeks, cells actually colonizedthe matrix and their morphology showed analignmentalong the fiber axis (Fig.1).
Our collagen threads constitute a promising *in vitro* 3D model to study the interplay between the complex supramolecular organization of the scaffold and the cell morphology/differentiation.

[1] *Agassant, J.-F. et al. Polymer Processing Extrusion Instabilities and Methods for their Elimination or Minimisation. Int. Polym. Process. 21, 239–255 (2006)*