3D Synovial Organoid Culture Reveals Cellular Mechanisms of Tissue Formation and Inflammatory Remodelling

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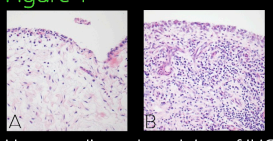
Introduction

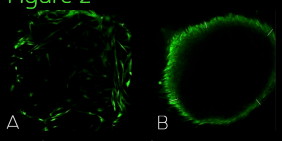
The synovial membrane is a distinctly organized structure with two layers: a densely packed lining layer that sits on top of a more loosely organized sublining layer (Fig.1A).
During the course of arthritis, the synovium becomes hyperplastic and demonstrates thickening of the lining layer and cellular condensation in the sublining layer (Fig.1B).
Using a 3D synovial organ culture system, we explore cellular mechanisms of synovial tissue formation and inflammatory remodelling.

Figure 1



Hematoxylin-eosin staining of IHC sections of OA (A) and RA (B) synovial tissues.

Figure 2



Confocal image of a single section from a one week old (A) & a four weeks old (B) synovial micromass. Green cell tracker dye was used to stain FLS.

Methods

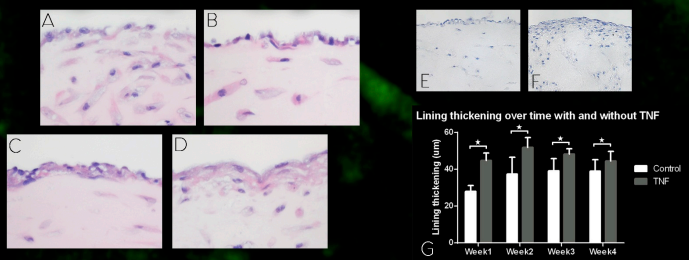
Fibroblast-like synoviocytes (FLS) derived from patients with rheumatoid arthritis (RA) were cultured in three dimensional Matrigel micromasses.
To mimic synovial inflammation, micromasses were challenged with TNF.
For histological analyses, micromasses were embedded in paraffin sections and were stained with hematoxylin and eosin (HE); Ki67 labelling was performed to identify proliferating cells.
Two-photon laser scanning microscopy was used to measure lining layer thickness during the culture period (Fig. 2).
3D confocal micrographs were analyzed using Imaris® Bitplane software. mRNA levels for various genes expressed in FLS were determined by qPCR.

Results

Synovial micromasses demonstrated thickening of the lining layer over time (Fig. 3A-D). When stimulated with TNF, cellular aggregation at the sublining layer and hyperplasia of the lining layer were observed (Fig. 3E-F). Statistical analyses determined lining thickening as a time-dependent ($P<0.001$) and TNF-dependent process ($P<0.01$) (Fig. 3G).

Figure 3

HE staining of IHC sections from 1, 2, 3 and 4 weeks old micromasses (A, B, C D, respectively) showing synovial lining formation. IHC of not-stimulated (E) and TNF-stimulated (F) micromasses depicting characteristic architectural changes. Graph representing lining layer thickening measurements over time with and without the presence of TNF (G).



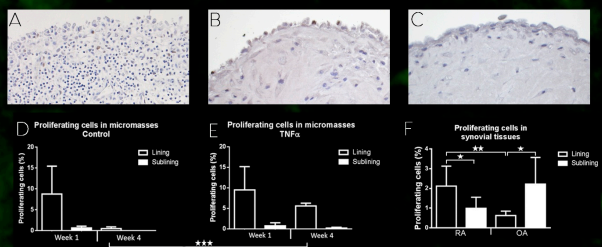


Figure 4 Ki67 labeled IHC sections from RA synovium (A) and non-stimulated micromasses, one and four weeks old (B, C, respectively). Graphs represent percentage of proliferating cells from the lining and sublining layer separately. They compare week 1 vs week 4 from control micromasses (D), TNF-stimulated micromasses (E) and synovial tissues from RA and OA patients (F).

In order to identify the origin of cells contributing to the thickening of the lining layer, proliferation studies were conducted (Fig.4 A-C). Intriguingly, in the early phase of the culture period, proliferation of cells in the lining layer was higher compared to the sublining layer (Fig. 4D). This proliferative activity, however, was no longer present in the late phase, after the lining layer was established (mature phase) (Fig. 4E).
In the presence of TNF, an increased number of proliferating cells in the lining layer was maintained for an extended period of time. This was consistent with higher rates of cellular proliferation in the lining in sections of RA synovial tissues when compared to OA synovial tissues (Fig. 4F).

During the course of lining/sublining layer maturation, mRNA expression levels of genes of interest were measured. qPCR data indicate that MMP1, MMP3, and IL-6 are differentially expressed during the early phase (one week old) and the mature phase (four weeks old) of the culture period. By contrast, lubricin, cadherin-11, CCL20 and STAT1 gene expression did not show a significant difference (Fig. 5).

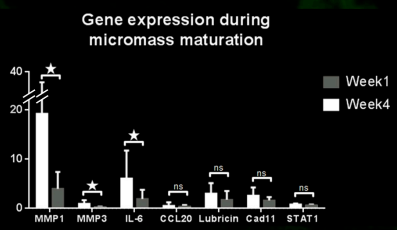


Figure 5

Gene expression in micromasses compared in Week 1 vs Week4.

Conclusions

The 3D synovial micromass culture spontaneously forms a tissue structure that strikingly resembles the lining/sublining architecture of the in-vivo synovial tissue. This process involves FLS proliferation as well as expression of genes that allow for tissue remodelling. In inflammatory conditions similar cellular programs are re-activated resulting in synovial lining hyperplasia and a pannus-like condensed mass of cells.

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