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Maximising Efficiency of Spatial Transcriptomics in Scleroderma: Challenges and Opportunities

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Scleroderma (SSc) is a chronic, multi-system, auto-immune mediated disease that impacts up to 240 million people worldwide [1]. Thickened, fibrotic skin is the hallmark of SSc. Due to the accessibility of the skin for biopsy, this has provided the opportunity to study SSc disease processes at a histopathological level with SSc skin biopsies found to have increased fibroblasts, a thickened cutis and immune cell infiltration [2,3]. RNA sequencing of whole biopsies and single-cell RNA sequencing have shed an unprecedent level of information on the complexity of gene signatures during distinct disease stages [4,5]. Further research is required to understand spatial gene expression and identify key cell types across the disease continuum to improve our knowledge of SSc development, prognostication and to identify potential therapeutic applications.

Spatial transcriptomics (ST) combines sequencing technologies and microscopic imaging to facilitate the study of gene expression from cells in their original space and physiological context [6,7]. This provides vital information on inter-cellular connections and their interactions within their own environment [7]. ST has also overcome the limitations of bulk RNA sequencing, allowing to filter the large transcriptome noise from keratinocytes and focus specifically on areas of interest and identify key cell types within the dermis. Despite the clear benefits of ST, the main limiting factor of this method is expense, costing thousands of pounds per slide. In order to achieve the required numbers for statistical analysis in an economical manner, building a tissue microarray (TMA), which arranges multiple different biological samples into a single paraffin block, could aid in overcoming this expense barrier.

TMAs have had an undisputed impact within the field of oncology. They have permitted the rapid assessment of samples from large patient cohorts and have been utilised in the identification of new oncogenes, such as the EMSY oncogene in breast cancer, and in the development of diagnostic assays [8]. Using TMAs for ST analysis in SSc has the potential to analyse large patient cohorts and yield breakthroughs of a similar calibre. In particular, understanding the infiltrating immune microenvironment in pre and early SSc skin would deepen our understanding of how SSc develops. Furthermore, the study and comparison of biopsies from the same patient before and after therapeutic intervention could provide us with direct reverse translation of the key processes involved in SSc pathogenesis and offer an objective assessment of benefit within diseased tissue. In addition, TMA-ST could offer affordable and valuable analysis of ex vivo treatment of precision skin cuts [9].

To produce a TMA, a punch biopsy of a solid core encompassing the tissue biopsy is taken from the original donor paraffin block and then precisely arranged within a new paraffin block called the recipient block [10]. Sections are then taken from the recipient block with multiple samples now being available for transcriptomic analysis. However, there are limitations to consider when attempting to create a TMA from skin biopsies. The skin has a specific orientation of epidermis through to the dermis and hypodermis. It is essential that such orientation be preserved during the creation of a TMA as all skin layers are involved in the pathogenesis of SSc. For example, the epidermis and its appendages have been shown to be

atrophied, fibrosis may be seen in both the papillary and reticular dermis and the subcutaneous tissue may be infiltrated by immune cells (namely lymphocytes, plasma cells and macrophages) [3].

Preserving skin orientation can prove to be technically difficult when preparing a TMA – organising the correct orientation and depth for multiple fixed samples whilst the wax is setting is an immense task. Combining the cores of paraffin embedded skin tissue within the recipient block is also challenging and it may result in the tissues being out of sync in depth. This can result in multiple slicing attempts required to obtain a final section that contains every sample and therefore can lead to the potential loss of valuable sections and/or regions of interest.

To summarise, ST analyses on skin biopsies from patients with SSc could potentially provide improved understanding of the complex pathophysiology of this disease, allowing the simultaneous analysis of interacting cells and their resulting effect on their transcriptomes within the dermal environment. However, due to technical difficulties previously discussed, we are currently limited to analysing four samples per slide. Of the four samples, some may become warped, rendering areas that are unsuitable for ST analysis. There are, however, commercial examples in which TMAs have been created from skin biopsies, showing that these limitations can indeed be overcome. For individuals with a research interest in SSc, lupus or dermatomyositis, an investment into procedures aimed at building TMAs for skin biopsies, with a focus of preservation of biopsy orientation, may be beneficial. Such an investment could vastly reduce costs associated with performing ST, allowing for multiple samples to be analysed and offering a more robust data set and hopefully enhancing our knowledge of SSc prognostication, progression and aiding in much needed new drug discovery.

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