SDS/DTT As a Novel Antigen Retrieval Method in Immunofluorescence

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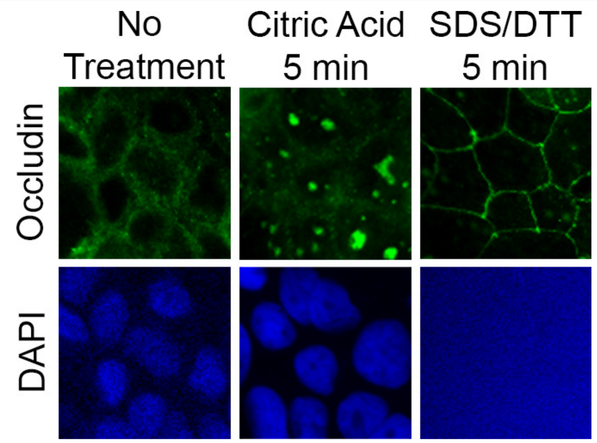
Introduction

- Immunofluorescence (IF) has useful implications in detecting co-localisation of molecular markers.
- Fixation with paraformaldehyde (PFA) usually retains the sample's morphology but has signal detection limitations. PFA-fixed samples are highly crosslinked and thus antibodies may not recognise the buried epitopes resulting in unsatisfactory staining.
- Antigen retrieval refers to techniques that reverse the masking of an epitope and restore epitope-antibody binding.
- In SDS-PAGE, heat, sodium dodecyl sulfate (SDS) and dithiothreitol (DTT) are used to break down the disulphide bonds, secondary and tertiary structures of the proteins.
- This project compares an established antigen retrieval method for histology using citric acid and heat to a novel method using SDS, DTT and heat.
- This poster also illustrates part of the optimisation process of this novel method and its application to antigen-detection in PFA-fixed samples by IF.

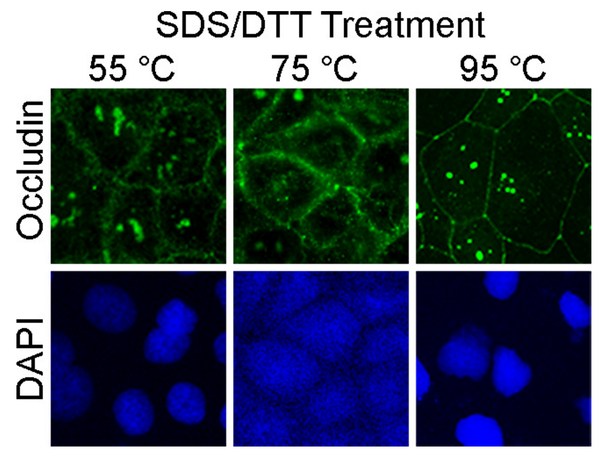
Methods

- Confluent Caco-2 cells were fixed with 4% PFA, and permeabilised with 0.1%Triton X-100 in PBS. Prior to blocking, cells were treated with aqueous solution containing either citric acid or SDS and DTT at high temperature.
- During the optimisation process, high temperature was tested with either microwaving or boiling samples in water bath containing respective solutions. Similar optimisations were also done to test the concentration of SDS and DTT.
- Then the cells were stained for the presence of tight junction proteins, such as occludin and ZO-1.
- The generalisability was also tested for the IF detection of claudin and cingulin.

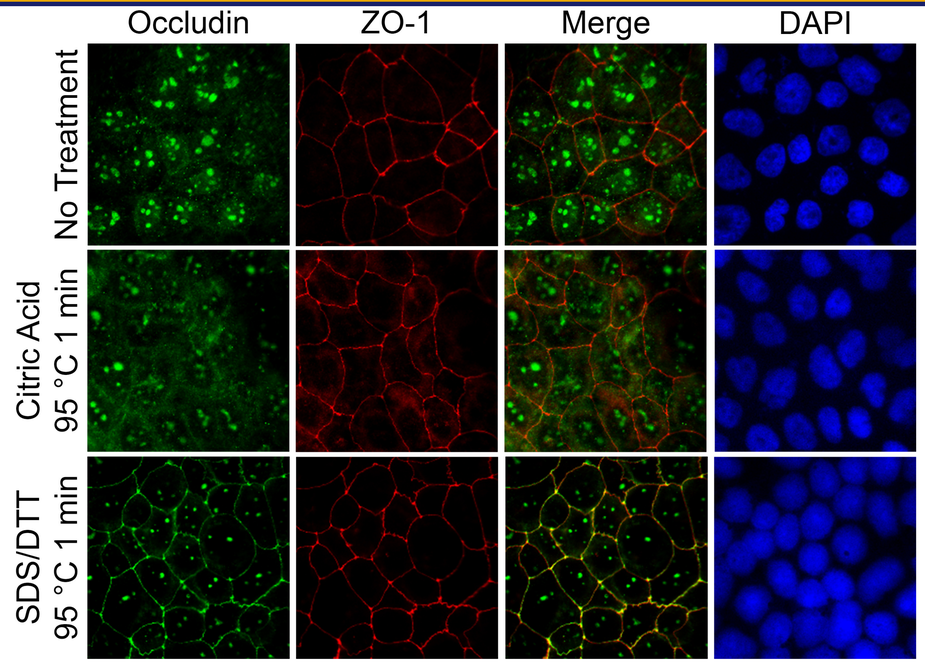
Results



▲ **Figure 1. Junctional staining improves with high heat in microwave, but cell nuclei disintegrate.** After PFA fixation and before blocking, Caco-2 cells were untreated, boiled for 5 minutes in aqueous solution with 0.1M citric acid or 1.3% SDS and 0.52% DTT using microwave. Then the cells were stained for the presence of occludin. This shows SDS, DTT and high heat as a potential method of antigen retrieval in IF staining.



▲ **Figure 2. Optimisation of Temperature: 95°C shows best junctional staining.** After PFA fixation and before blocking, Caco-2 cells were boiled for 1 minute in 1.3% SDS and 0.52% DTT at various temperature in water bath. Then the cells were stained for the presence of occludin.



▲ **Figure 3. Optimised SDS/DTT treatment unmasked occludin epitopes in tight junctions.** After fixation and before blocking, Caco-2 cells were untreated, boiled for 2 minutes in aqueous solution with 0.1M citric acid or 1.3% SDS and 0.52% DTT. Then the cells were stained for the presence of occluding and ZO-1.

Discussions

- While citric acid treatment showed little effect compared to no treatment, cells treated with 1.3% SDS and 0.52% DTT at 95°C for 1 minute improved junctional staining of occludin, while maintaining the cell nucleus and ZO-1 staining, which is our optimised antigen retrieval method.
- Further testing will verify other immunostaining patterns, such as endosome markers and membrane trafficking.

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