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Scope and Limitations of Current 3D Spheroid Culture Screening Systems

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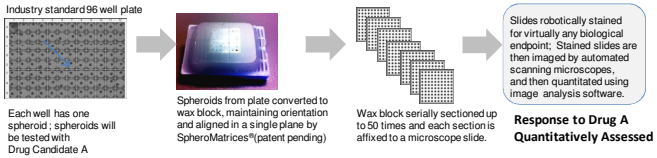
Summary

We compared outputs for immunofluorescence (IF) staining, imaging and analysis of spheroids using SpheroMatrices™ spheroid tissue microarray technology (TMA) with an existing whole mount confocal imaging technology (Yokogawa CV7000) (Fig 1). Brain microphysiological system (BMPS) spheroids and BMPS seeded with GFP-tagged glioblastoma cells (gBMPS) at 4, 5 ,6,7 and 8 weeks (wk) differentiation were IF stained with the neuronal marker NeuN or GFP, respectively, in parallel using two different methods. For the TMAs, microscope slide sections of the arrays were stained using standard IF staining methodology, and for the CV7000 system BMPS were immunofluorescence stained in a **whole mount** format (Fig1). NeuN staining of TMA sections showed characteristic nuclear staining of this RNA splicing protein whereas the staining of whole mount prep did not show any nuclear staining. Imaging of the TMA, performed using a Zeiss 700 confocal microscope, showed complete imaging right through the spheroid (Fig 2 A) whereas the CV7000 (spinning disc confocal) system was only able to image the outer areas of the spheroid (Fig 2 B) due to light scattering/quenching preventing in depth imaging. We performed automated image analysis across the TMA with bespoke scripts using Image J (FIJI) software and this showed that there was a significantly ($P<0.001$) higher intensity of NeuN staining in the 8w BMPS nuclei compared to the 5w (Fig 2 C, D) consistent with NeuN being a marker for fully differentiated neurons. GFP IF staining and image analysis of the gBMPS showed there was a time-dependent increase in the number of tumour cells relative to the normal neuronal cells indicating growth of the 'tumours' over time, Fig 3 and Fig 4A. The number of non-tumour (normal neuronal cells) in the gBMPS did not increase with time reflecting their post-mitotic status, Fig 4B.

TMA Imaging (SpheroMatrices™)



Whole Mount Confocal Imaging (Yokogawa CV 7000)

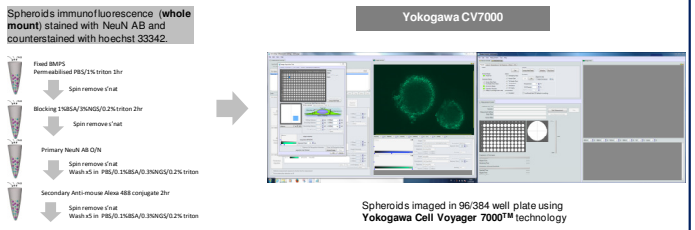


Fig 1. Methods for imaging and analysis of spheroids using SpheroMatrices spheroid tissue microarray (TMA) technology versus whole mount confocal imaging technology (Yokogawa Cell Voyager 7000).

Results

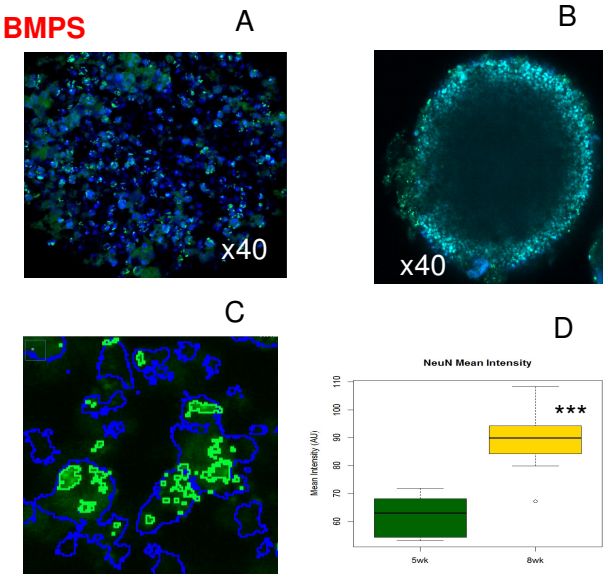


Figure 2. Imaging and analysis of NeuN IF stained BMPS spheroids with TMA (SpheroMatrices) and whole mount confocal (Yokogawa CV7000): (A) TMA NeuN IF staining; (B) NeuN whole mount stain (Yokogawa CV7000) ; C: TMA NeuN image analysis mark up; (D) TMA NeuN IA data – NeuN staining intensity significantly higher at 8w compared to 5w, ***p<0.001, n=10. Note : Blue is hoechst 33342 nuclear counterstain.

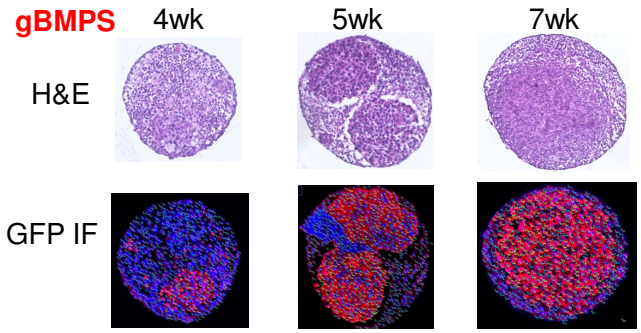


Fig 3. Imaging and analysis of H&E and GFP IF stained gBMPS on parallel sections of the TMA. Top Panels: H&Es – note the glioblastoma (tumour) cells are more eosinophylic than the normal neuronal cells. Bottom panels: GFP IF stained (red) glioblastoma cells. Blue is hoechst 33342 nuclear counterstain

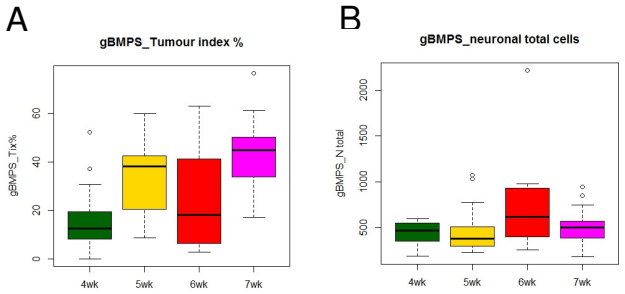


Figure 4. Box plots of GFP IF stained gBMPS TMA image analysis data. A: tumour growth index = glioblastoma (GFP+) cells/total spheroid cells (glioblastoma + normal neuronal)x100%; B: total neuronal cells (GFP-) . The data was generated using a bespoke script using Image J (FIJI) software. The ends of the boxes are the upper and lower quartiles (interquartile range) and the black line shows the median, n=20)

Conclusions

- NeuN IF staining of TMA sections was able to visualise the characteristic nuclear staining of this RNA splicing protein whereas whole mount staining could not.
- The difference in staining was attributed to the fact that the staining of fixed tissue with the NeuN antibody requires heat induced epitope retrieval (HIER) which was not possible with whole mount staining of spheroids.
- TMA IF staining/imaging was able to visualise and quantify staining throughout the whole body of the spheroid whereas whole mount staining and imaging on the CV7000 could not due to light scattering/quenching.
- The TMA technology was thus able to quantify the extent of neuronal differentiation in the BMPS and the growth of glioblastoma 'tumours' in the gBMPS as reflected by NeuN and GFP staining, respectively.
- As the TMA retains the organisation of spheroids cultured in a 96 well plate format this approach is compatible with medium throughput drug screening.

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