HIGH-THROUGHPUT ELECTROSPUN AND LITHOGRAPHIC PLATFORMS FOR ADVANCED BIOIMAGING OF MECHANOTRANSDUCTIVE-RELATED EVENTS IN GLIOBLASTOMA

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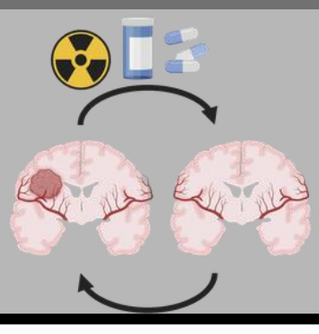
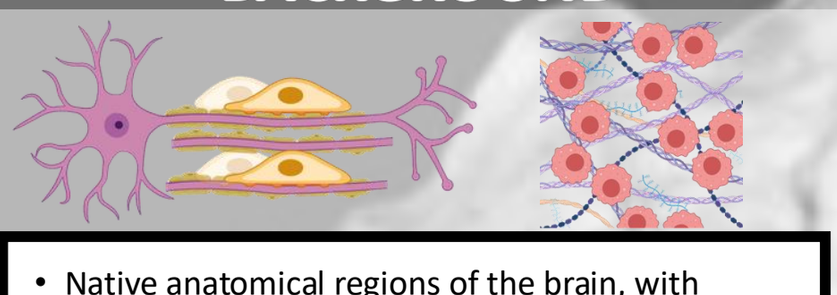
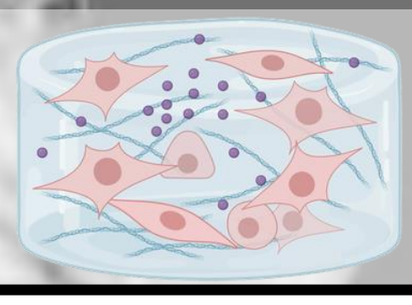
BACKGROUND

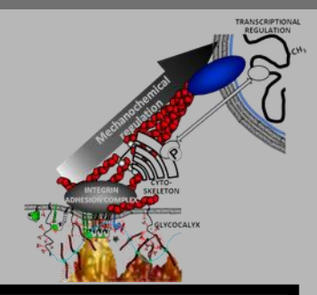


- Glioblastoma is a lethal primary brain tumour with near 100% recurrence [1]
- Novel approaches are critical to screen therapeutic approaches and understand glioblastoma cell behaviour *in vitro*



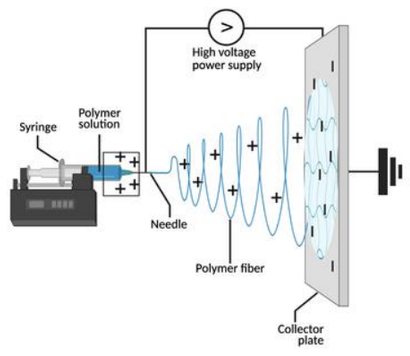
- Native anatomical regions of the brain, with aligned ECM, such as white matter tracts and blood vessels, or unaligned ECM, such as brain parenchyma, are exploited by invading glioblastoma cells
- The orientation and 3D nanoarchitecture of these features can determine cell migration directionality, mechanotransductive, and pathophysiological cell responses [3,4]



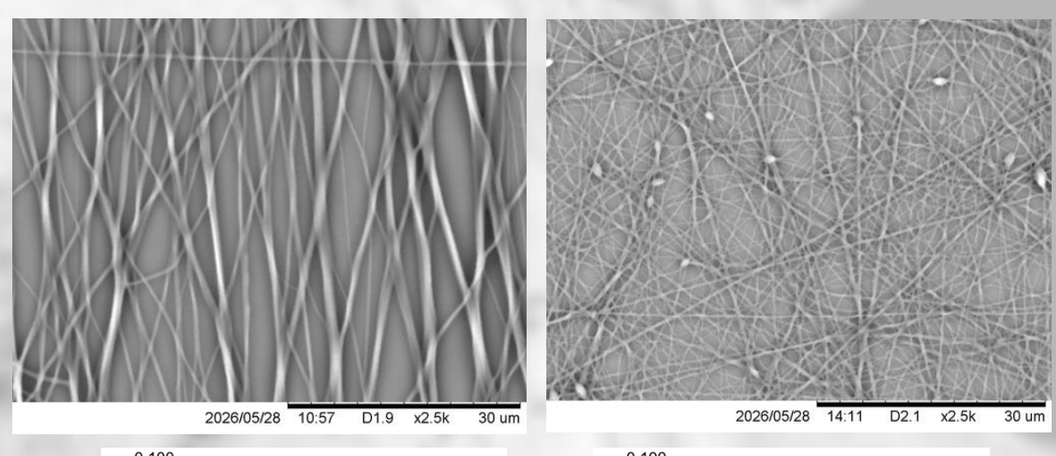


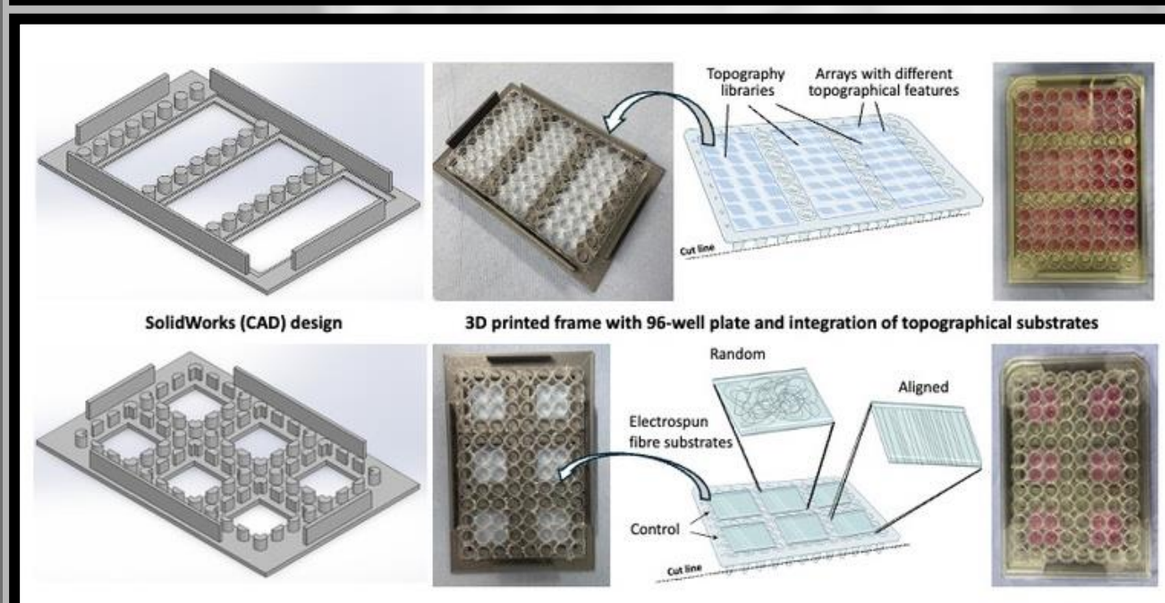
- Many *in vitro* models lack the ability to study cell responses to physiologically relevant topography [5,6]
- Electrospinning approaches can produce customisable topographical substrates to model aspects of brain and tumour microenvironments

FABRICATION OF SUBSTRATES BY ELECTROSPINNING



- Fibrous gelatin substrates were produced using electrospinning
- Fibre orientation was evaluated using scanning electron microscopy
- Fibre orientation determined within Fiji's directionality plugin





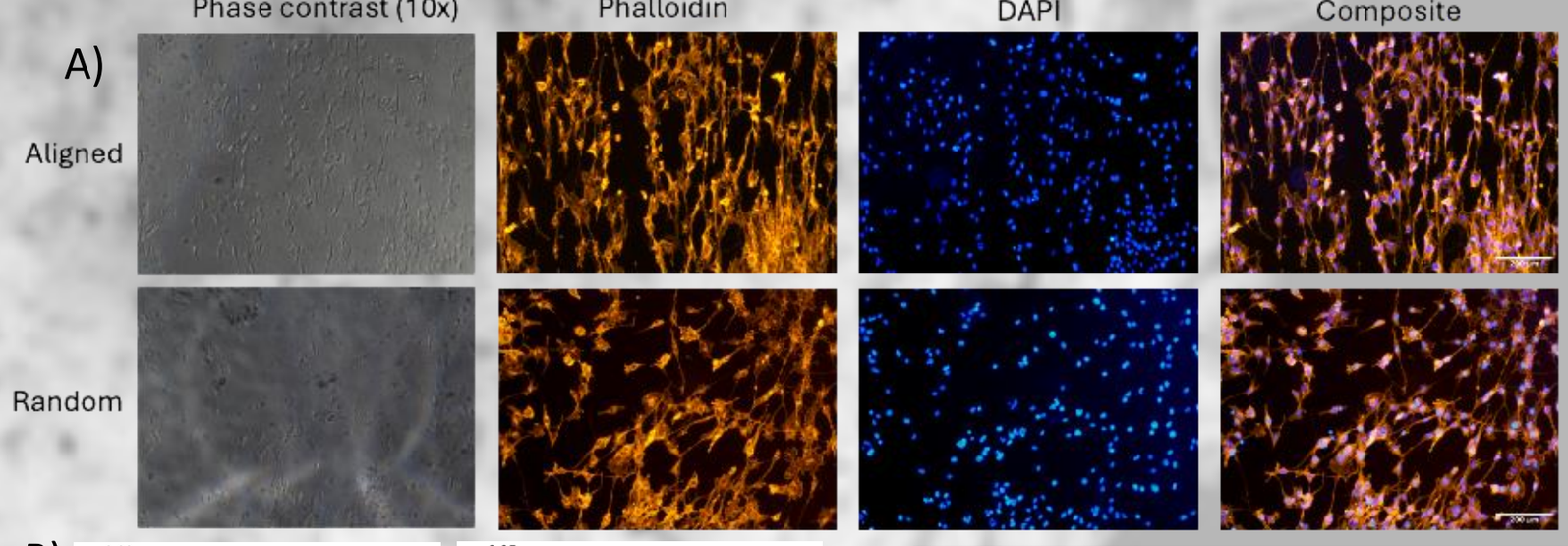
- Versatile platform compatible with plate readers and microscopes

Characterisation of electrospun substrates. A) SEM migrograpghs of aligned (left) and random (right) gelatin substrates. B) Directionality histogram of aligned (left) and random (right) using fourier components directionality analysis on FiJI (N = 3).

SIM-BASED VALIDATION OF CELLULAR RESPONSES TO TOPOGRAPHICAL CUES

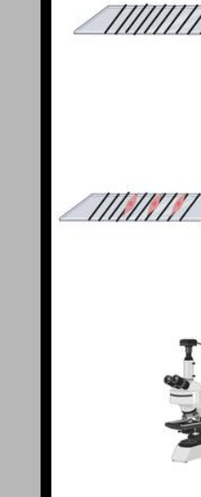
METHODS

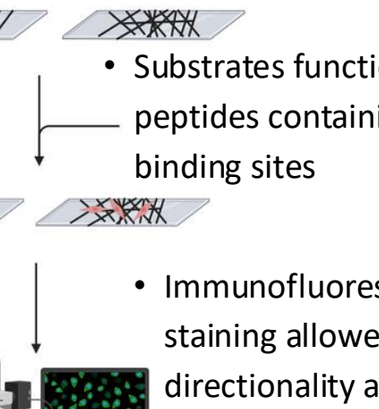
- Patient-derived GB cells seeded onto random/aligned substrates
- Substrates functionalised by peptides containing integrin binding sites
- Immunofluorescence staining allowed cell directionality and morphology evaluation by Structural Illumination Microscopy (SIM)-based recordings



Glioblastoma cell morphology is influenced by substrate orientation and biochemistry. A) Glioblastoma cells seeded onto aligned and random electrospun substrates for 72h . B) Cell directionality analysis on aligned/random substrates following 72h in culture N= 3. B) Cell area measurements on aligned/random substrates following 72h in culture (20x magnification) N=1 (preliminary).

Ongoing Work





Topographical substrates produced by lithography.

- Substrates produced using lithography are highly reproducible and customisable.
- Glioblastoma cell morphology and mechanotransductive events will be evaluated on gratings of varying widths. In collaboration with Prof. Nikolaj Gadegaard (UoG).

References

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