

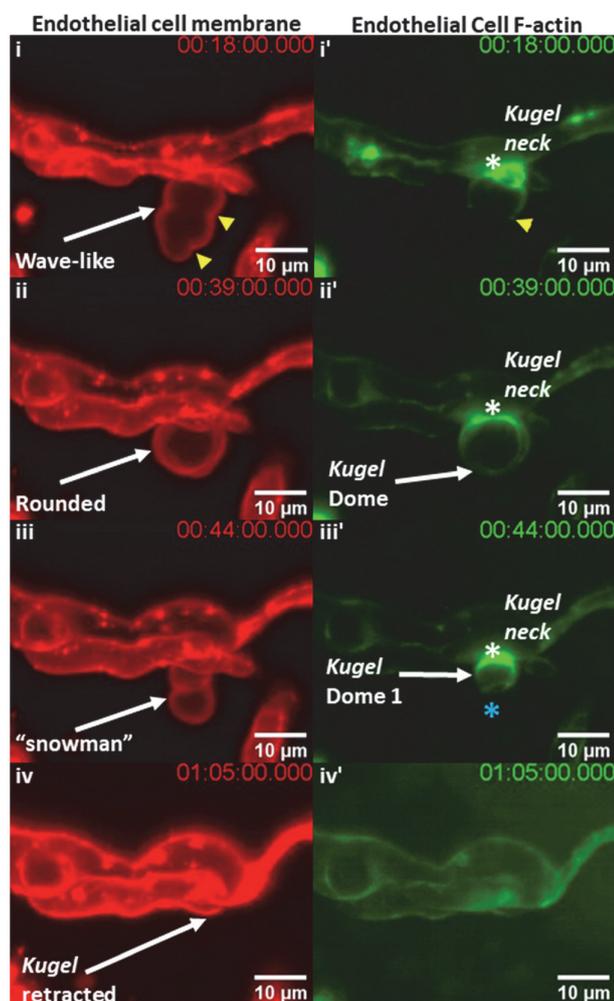
BS42 CHARACTERISING SPATIOTEMPORAL MEMBRANE AND F-ACTIN DYNAMICS IN KUGELN, A NOVEL ENDOTHELIAL MEMBRANE BEHAVIOUR IN THE ZEBRAFISH CEREBRAL VASCULATURE

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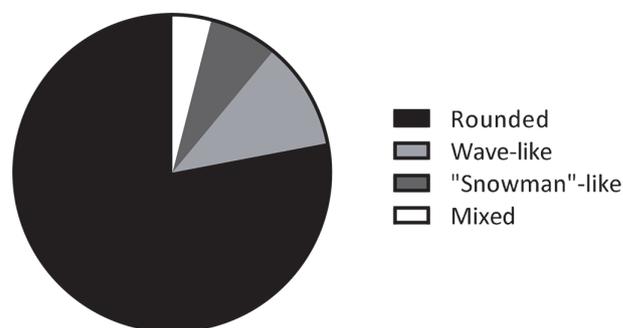
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Introduction Kugeln are a recently characterised endothelial cell (EC) membrane behaviour in zebrafish, exclusive to the cerebral vasculature and of unknown function. Kugeln are highly dynamic structures that alter their shape and size throughout their lifetime. Early studies revealed that the formation of kugeln is regulated by filamentous actin (F-actin) cytoskeletal dynamics, with F-actin localised predominantly at the kugel neck. However, the spatiotemporal dynamics of F-actin recruitment and redistribution remain to be studied. Therefore, we characterised kugel F-actin dynamics in relation to the kugel membrane in the zebrafish cerebral vasculature, using *in vivo* time-lapse light sheet imaging.

Methods Double-transgenic RFP-tagged EC membrane Tg(kdrl:HRASmCherry)s916 and GFP-tagged EC F-actin Tg(fli1a:Life-Act-mClover)sh467 3–4 dpf embryos were anaesthetised and imaged with a Zeiss Z.1 light sheet fluorescence microscope



Abstract BS42 Figure 1 Mixed type Kugel membrane (red) behaviour transitioning between wave-like, rounded and 'snowman'-like behaviours and characteristic F-actin dynamics (Green)



Abstract BS42 Figure 2 The proportion of distinct kugel behaviours observed in the 3–4dpf zebrafish midbrain vasculature

to capture 2-hour time-lapse recordings (time interval – 1 minute) of up to 100µm x 100µm regions of interest at 1µm z-intervals within the midbrain. Raw data was processed with FIJI (v1.51) using custom macro plugins created by our group. Each kugel observed was analysed to evaluate the kugel EC F-actin and EC membrane dynamics over time.

Results We analysed 45 kugeln from 15 zebrafish embryos (3 experimental repeats) and observed three distinct kugel membrane behaviours. These included rounded kugeln where the kugel membrane maintains a spherical shape, a 'snowman'-like behaviour where spherical kugeln domes occur on top of one another, and an undulating wave-like kugel membrane behaviour (Figure 1). A fourth, mixed kugel behaviour was also observed, where the kugel membrane dynamically transitions between the previously defined kugel membrane behaviours (Figure 1). 78% of the kugeln exhibited the rounded morphology and the remaining 22% displayed either the wave-like, 'snowman' or mixed behaviours (Figure 2). Each behaviour was associated with a distinct pattern of F-actin dynamics. F-actin in rounded kugeln was initially localised to the kugel neck and subsequently to the top of the dome. In the wave-like kugel behaviour, F-actin was localised to the kugel neck throughout its lifespan. In the 'snowman'-like kugeln, F-actin was predominantly localised at the neck of each successive kugel dome.

Conclusion We have further elucidated the spatiotemporal dynamics of kugel behaviour and linked discrete kugel membrane behaviours to specific patterns of F-actin rearrangement. These results provide more insight into the cytoskeletal mechanisms of kugel formation, maintenance and demonstrate the complexity of kugel behaviour.

BS43 PRO-ANGIOGENIC INJECTABLE SELF-ASSEMBLING PEPTIDE HYDROGEL AS A DELIVERY VEHICLE FOR CARDIAC CELL THERAPIES

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Introduction Cardiac cellular therapies offer a potential solution to restore cardiomyocyte populations post myocardial infarction (MI). However, when injected directly into the diseased heart only 1% of cells survive due to mechanical displacement and the ischemic nature of the heart post MI. Innovations in bioengineering have led to the development of