Selective cell propagation *via* laser micropatterning of a thermally-activated hydrogel

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Introduction

- A significant challenge in the development of monoclonal antibody therapeutics is the **production of adequate material for *in vitro*, *in vivo* and clinical testing**.
- Producer cells, including hybridomas and CHO cells have highly heterogeneous antibody expression and secretion[1]. As a result, additional screening is required to establish cell lines that **maximize productivity**.
- Established methods, such as limiting dilution and colony picking require significant wait times before antibody secretion can be quantified by ELISA[2] or colonies to be sufficiently large for extraction[3].
- Here, we developed the Selective Laser Gelation (SLG) method[4], which uses laser micropatterning to selectively arrest the growth of undesired cells in methylcellulose media. We demonstrate that arresting the growth of low-yield IgG4 secreting CHO cells in a mixed population results in a production advantage that is preserved over an extended period of culture.

Objectives

- To develop the experimental apparatus for selective propagation of cells in methylcellulose
- To demonstrate that SLG can improve overall yield of antibody production

Principles of Selective Laser Gelation (SLG)

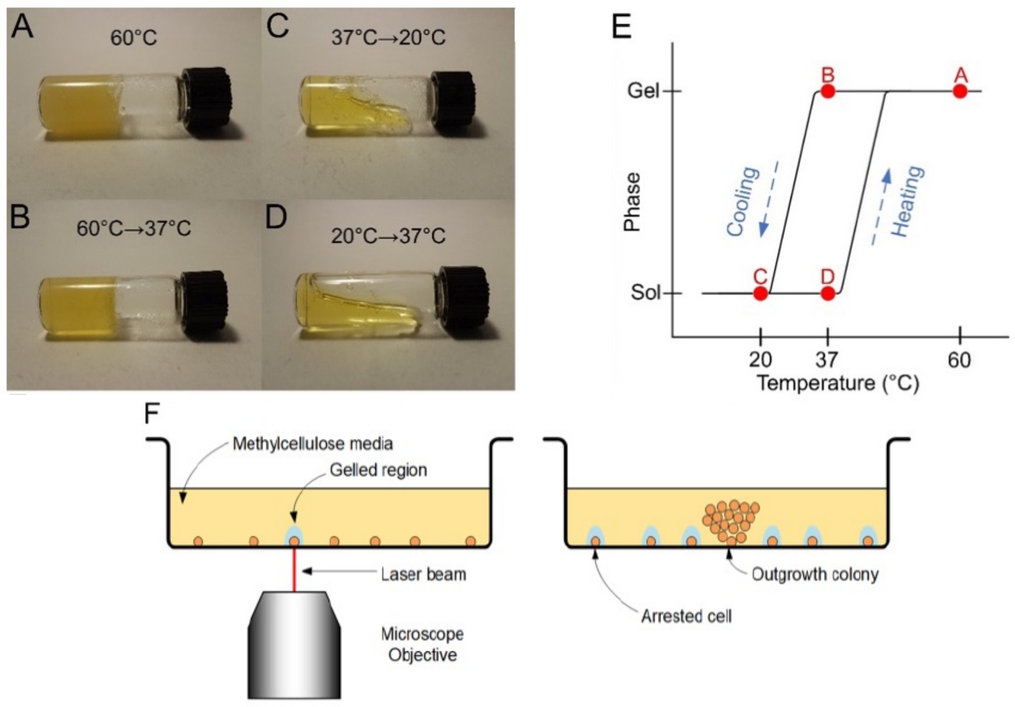

Figure 1. Principles of selective laser gelation.
A-E: Inverse solution-gel transition property of methylcellulose. A: 2% methylcellulose is heated from 20 °C to 60 °C, which turned the solution to gel. B: The gel is retained after cooling the vial to 37 °C. C: Further cooling to 20 °C resulted in the dissolution of the gel. D: The solution phase is retained after heating the solution back to 37 °C. E: Plot of methylcellulose phase changes with temperature. F: The SLG mechanism, where localized laser heating of methylcellulose enables hydrogel encapsulation of unwanted cells to stop their growth, while the rest of the cells continue to proliferate.

Validation of the SLG process

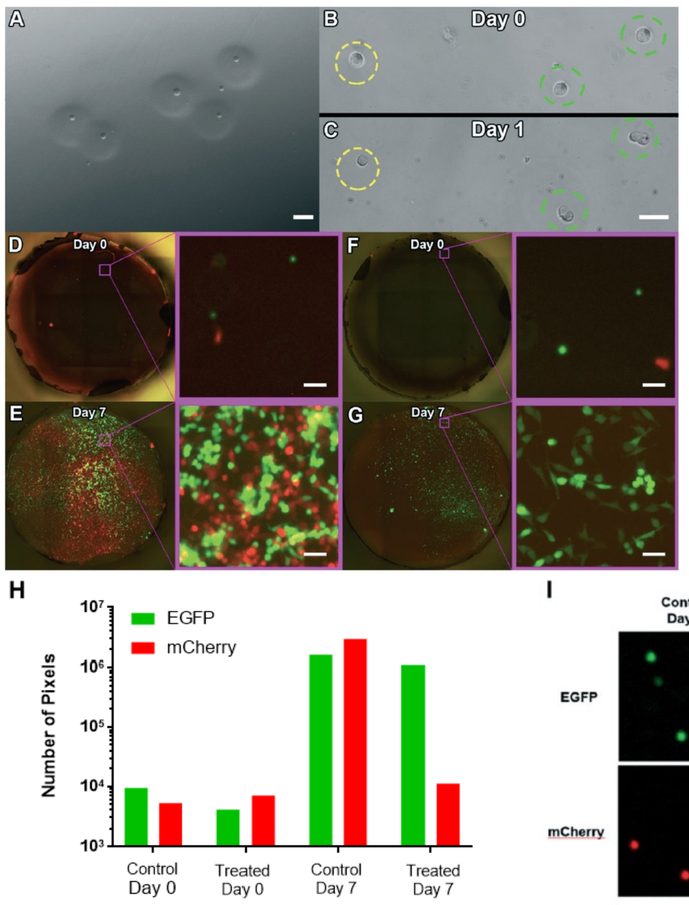

Figure 2. SLG process validation.
A: B13-24 CHO cells in methylcellulose after SLG. Gelled spots appear as faint circles when imaged in phase contrast.
B,C: Growth of SLG-treated cell (yellow broken circle) is restricted, while neighbouring untreated cells proliferated (green broken circle).
D,E: Co-culture of UM-UC3-mCherry (red) and UM-UC13-EGFP (green) cells over 7 days.
F,G: SLG was used to eliminate the UM-UC3-mCherry cells and only the UM-UC13-EGFP cells proliferated after 7 days.
H: Pixel count of the mCherry and EGFP cells in control and SLG-treated wells.
I: UM-UC3-mCherry and UM-UC13-EGFP cell morphology and density on days 0 and 7 in the control and SLG-treated wells.
(Scale bars = 50 μm)

Experimental apparatus

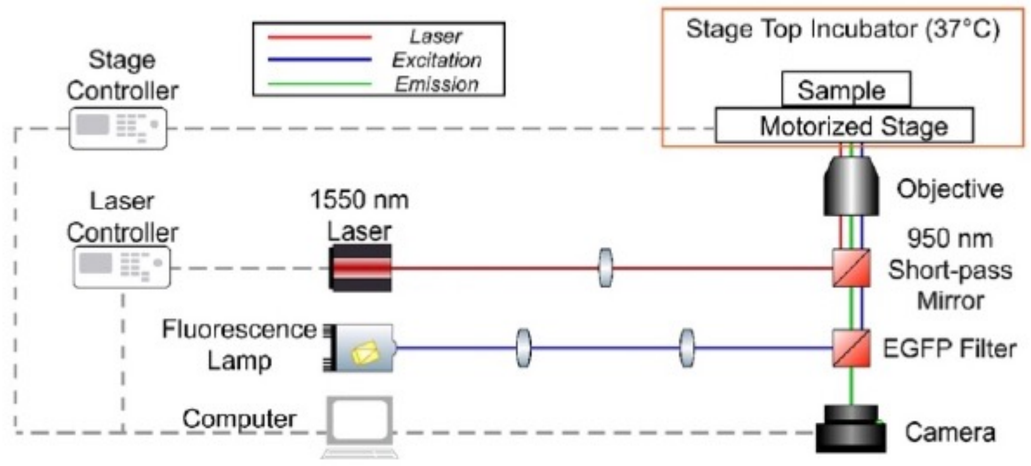

Figure 3. Laser patterning and imaging apparatus. Samples are imaged using a standard inverted fluorescence microscope and laser patterned using a 1550 nm laser.

Process for selection of high-producing CHO cells

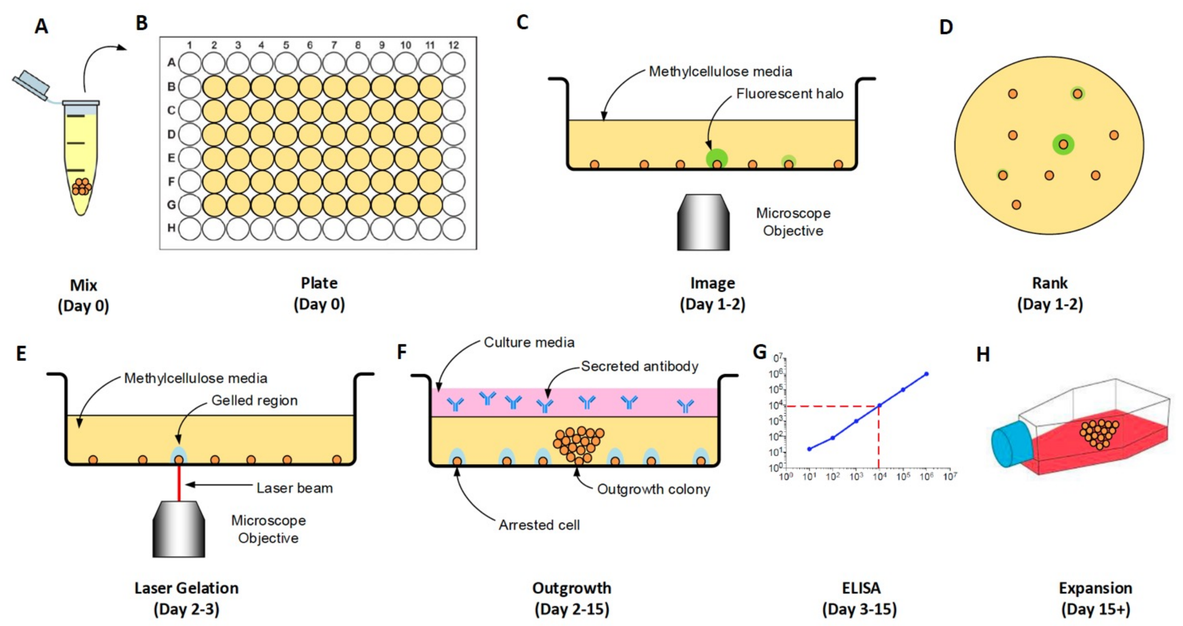

Figure 4. Process for selecting high-producing CHO cells. A: CHO cells are suspended in media with 1% methylcellulose and 1% FITC-labeled anti-human IgG (H+L) to detect secreted IgG4. B: The cell suspension is plated into a 96-well plate. C: Wells are imaged in brightfield and fluorescence. D: Each colony is ranked based on antibody secretion per cell. E: SLG encapsulates all low-producing cells in hydrogel. F: Wells are supplemented with liquid media for proliferation of remaining cells. G: Secreted antibody in the supernatant is quantified *via* ELISA. H: Culture is expanded in large flasks.

Results

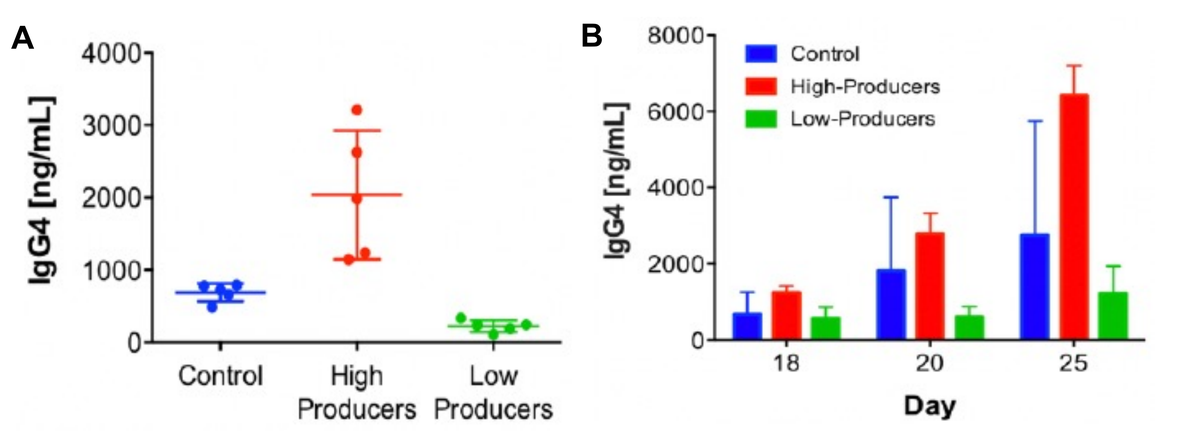

Figure 5. ELISA-based assessment of antibody secretion. A: Measurement of secreted IgG4 concentration in supernatant collected from 96-well plate on day 15 (N = 5). B: Increased IgG4 production is maintained by high-producing cells during expansion over 25 days into T-25 culture flasks.

Conclusion

The SLG method enables early screening for high producers at the single cell level and selective propagation of high-producing CHO cells. In doing so, timelines for isolation of candidate producer clones could be significantly reduced. More generally, the SLG method can be used to enrich cell populations for phenotypes of interest identified by imaging.

References

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