

Enhanced and homogeneous oxygen availability during incubation of microfluidic droplets.

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Abstract

Droplet microfluidic-based cell screening has the potential to surpass time- and cost efficiency of established screening platforms by several orders of magnitude, but so far lacks sufficient and homogeneous oxygen supply for large droplet numbers ($>10^6$), which is a key parameter affecting metabolism and growth of encapsulated cells. Here, we describe and validate an approach based on continuous carrier oil recirculation that ensures enhanced and homogeneous oxygen availability during mid and long-term incubation of picoliter droplets retained in a 3D-printed storage device. Using biotechnologically relevant microorganisms, we demonstrate that improved oxygen transfer results in three to eleven-fold increased biomass and highly elevated protein production with minimal inter-droplet variation. In fact, obtained yields are comparable to those achieved in conventional cultivation devices, so that screening strategies commonly applied in microtiter plates

or shaking flasks can now be scaled down to pL-droplets, which offer highly enhanced throughput. In contrast to mere single-cell screening, this approach allows monoclonal cell and metabolite accumulation inside droplets resulting in elevated read-out signals and reduced variability associated to stochasticity in gene expression. Additionally, the range of screening strategies is broadened, since screening for increased biomass yields or mining for microbial natural products from complex environmental samples can now be targeted with pL-droplets. This development substantially improves the robustness and versatility of droplet-based cell assays, further consolidating pL-droplets as a powerful ultrahigh-throughput experimentation platform.

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