CFD Simulation of Small-Scale Single-use Stirred Tank Bioreactors; Comparisons and Perspectives

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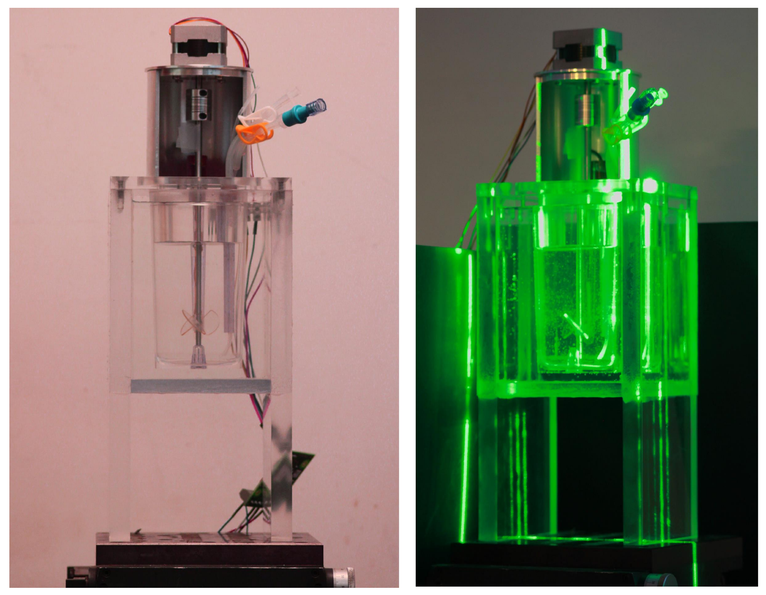
Background Emerging T cell therapies offer alternative cancer treatment options but bring new manufacturing challenges. Importantly, the need for sterile, single-use, bioreactors able to generate clinically useful cell yields. Stirred tank reactors (STRs) are mechanically simple, and can be manufactured cheaply as disposable therefore well suited for cell therapy applications. However there is little understanding of the fluid stresses and transport patterns in small scale STRs, and therefore their feasibility for T cell manufacture. Our aim was to build, validate and compare computational fluid dynamic (CFD) models of three commercially available, small scale (250ml), disposable STRs, in view of previously published STR T cell culture data.

	ambr® 250 modular	Eppendorf BioBLU® 0.3c	Corning® 500 Disposible
Vessel Radius (mm)	28	32	45
Impeller Radius (mm)	15	16.5	26.6
Rotational Speed (RPM)	150	136	85
Tip Speed (mm/s)	236	236	236
Fill Volume (mls)	250	250	410
Type of impeller	45 degree pitched blade	45 degree pitched Elephant Ear	Rushton with magnetic pill
Impeller drive	Mechancial coupling at lid	Magnetic coupling at lid	Magnetic coupling at base
Impeller blade height (mm)	18.5	21.5	60
Base to impeller centre (mm)	27.5	16	40
Fluid immersed features	3	6	0
Mesh Min size (mm)	0.05	0.05	0.05
Mesh Max size (mm)	2	2	2
Elements	2,896,477	1,401,599	3,697,876
Nodes	572,408	306,644	974,685

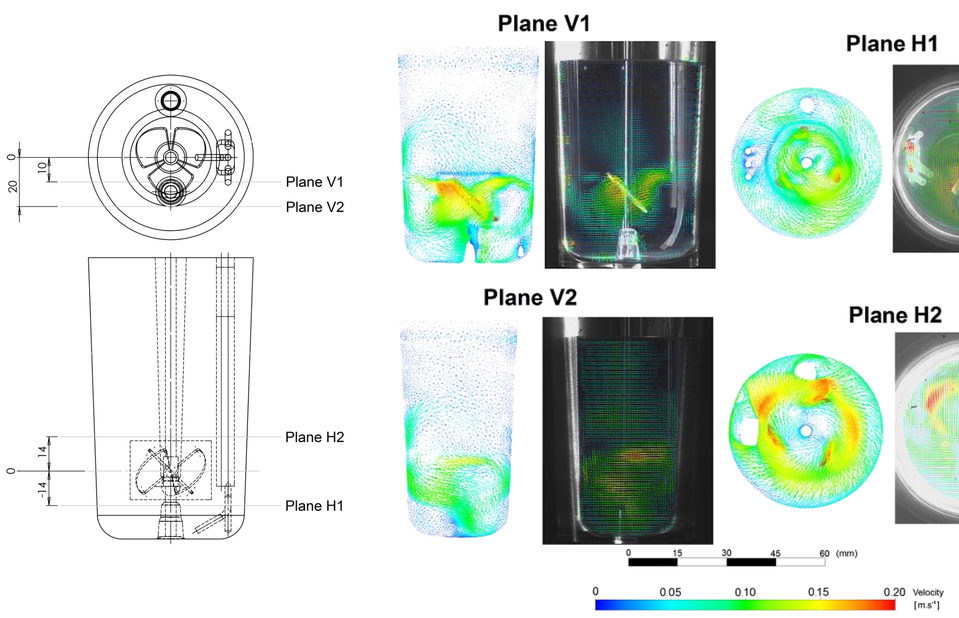
Table 1: Geometric, dynamic and mesh parameters for the three disposable vessel models; the ambr® 250 modular, Eppendorf BioBLU® 0.3c, and the Corning® 500 Disposable

Figures 1; CAD models of the three simulated disposable vessels, including an example geometry development process of the ambr® 250 modular vessel from CAD, to a meshed model of the vessel's inner volume

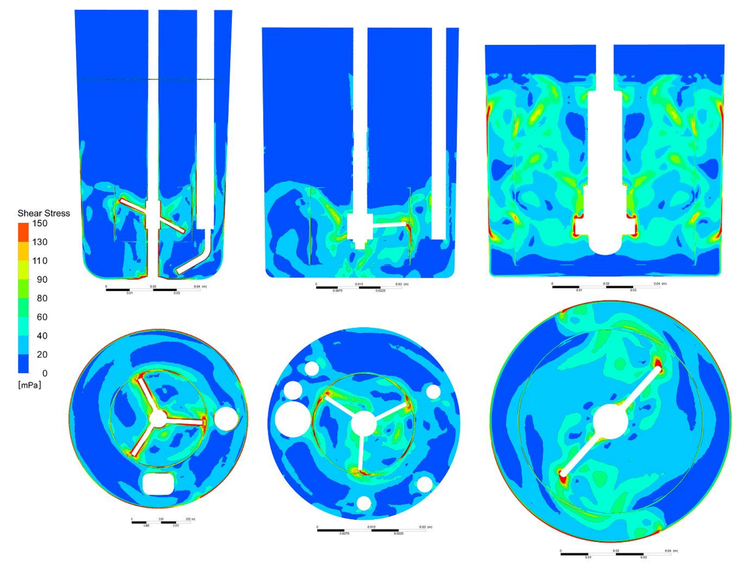


Figures 2; For PIV validation study, a replica of the ambr® 250 modular vessel was constructed inside a raised water bath (left) for PIV laser imaging (right)

Method Computer generated geometries were obtained from the STR provider, or from direct measurement of the vessel. As impeller diameters ranged between 30-53mm, rotational speed were adjusted to keep all tip speed at 236mm/s, (Table 1). As no disposable spinner flask option was found, a 500ml Corning disposable vessel was used. The ambr and Eppendorf vessels were simulated with a 250ml fill volume, and the Corning vessel with 410ml. The ambr® 250 modular vessel was used as an exemplar model with which to validate the CFD methodology. A replica was built and laser-derived fluid fields (PIV) were acquired for model validation, (Figure 2). Transient simulations were run until a quasi-steady state was achieved, and residuals were maintained below 10^{-3}. Velocity and sheer stress fields were compared, and the dynamic fluid structures' abilities to wash out the base of the vessel to keep cells in suspension were assessed.

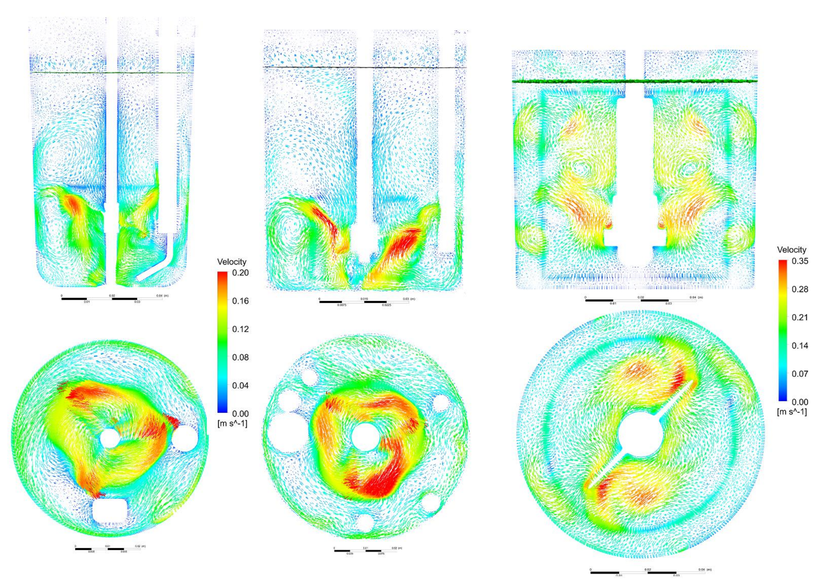


Figures 3; PIV and CFD comparison for CFD model validation in the ambr® 250 modular case. Image pairs consist of a CFD simulation velocity field (left of every pair), and a photo of the experimental vessel with the experimentally derived velocity fields (right of every pair)

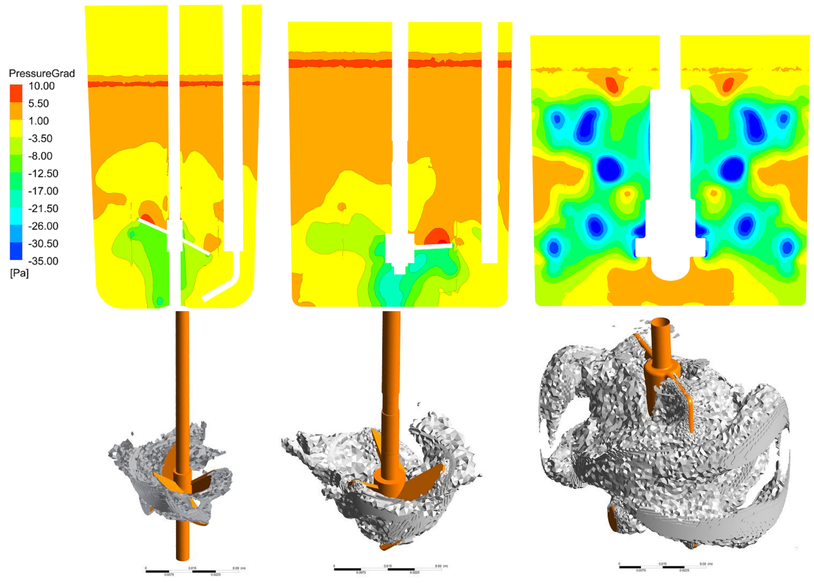


Figures 5; Comparisons of the shear stress field in both side elevation (top) and top view (bottom) between the ambr® 250 modular (left), Eppendorf BioBLU® 0.3c (middle, and the Corning® 500 Disposable (right)

Results The PIV and the CFD results showed high similarity in the exemplar model (Figure 3), thus validating the simulation strategy. Similar trends in dynamic fluid structures were observed in the two pitched blade impeller vessels (Figure 4). Due to the small space between impeller tip and vessel wall, a toroidal structure was shed from the impeller tips with average shear stresses generally under 100mPa (Figure 5). With kinetic energy focused in the toroids, mixing at the top surface or at the base of the vessel was reduced. However, impeller proximity to the base encourages base washout with the two pitch blade vessels creating a clear suction created under the impeller (Figure 6 above). The Corning vessel on the other hand generated high bulk rotational velocity with the prominent component in the tangential direction. Additionally the broad Rushton style impeller blades shed vortices from both the top and bottom outer corners, which eventually merge into a trailing loop structure, (Figure 6 bottom, right). These interactions between vortices generate high rates of change of velocity/shear.



Figures 4; Comparisons of the velocity field in both side elevation (top) and top view (bottom) between the the ambr® 250 modular (left), Eppendorf BioBLU® 0.3c (middle, and the Corning® 500 Disposable (right)



Figures 6; Comparisons of the adjusted pressure (above, i.e. weight contributions removed), fields to demonstrate the varying regions of suction and higher pressure, and the high velocity regions (below, >0.14 m/s, or 60% of tip speed) between the vessels.

Conclusion Despite high variation in local velocities in small scale STRs, we previously observed high cell yields making simple geometry STRs potential options for both allogeneic and autologous manufacturing. Nevertheless, bioreactor design focusing on either base washout to maintain cells in suspension, or high sheer rates to trigger cell differentiation can be guided by this work. There is potential for relatively simple modifications to augment these effects, but will require lab-based testing to observe the effect upon cell growth/quality. Certainly immunotherapy manufacture may be targeted to maximise cell yield, potency, and maintain critical quality attributes.

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