SPIRA MIRABILIS ENHANCED DENSITY GRADIENT CENTRIFUGATION
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ABSTRACT
The centrifugal microfluidic “lab-on-a-disc” paradigm is particularly suited to the pre-conditioning of whole blood. In this work we define the geometry of sedimentation chambers using the Spira Mirabilis (equiangular spiral) and so enhance the Boycott Effect and increase the speed at which blood may be stratified using a density gradient media. We find that using the Spira Mirabilis defined chambers can increase the speed of stratification by up to 25% compared with equivalent linear chambers.

INTRODUCTION
First defined and studied by Descartes, the equiangular spiral was christened the Spira Mirabilis by the famous mathematician Jacob Bernoulli [1] who was fascinated by its self-similarity; though the size of the spiral increases its overall shape does not and any radial line extending from the center of the spiral is at a constant angle to the tangent of the spiral at the point of intersection (Fig. 1). The Spira Mirabilis, also called the logarithmic or equiangular spiral, frequently appears in nature, e.g. as the shape of the arms on spiral galaxies [2], the flight paths of raptors approaching their quarry [3], the rainbands of tropical cyclones [4], the rainfalls of tropical cyclones [4] and the patterning of biological structures such as Nautilus shells [1] (Fig. 1a). In this work we for the first time use this property of the equiangular spiral in combination with density gradient media to synergistically enhance the Boycott effect and thus significantly increase the speed of separation of Peripheral Blood Mononuclear Cells (PBMCs) on a centrifugal lab-on-a-disc platform.

The Boycott effect was first discovered in 1920 [5] when it was noticed that erythrocytes sediment from whole blood at a faster rate when vessels were inclined at an angle as opposed to oriented vertically. A qualitative explanation of the Boycott effect is that particles sedimenting in angled chambers hug the lower walls. Therefore the collective, drag-based effects of displaced up-flow on sedimenting particles are attenuated [6].

As lab-on-a-disc platforms have been developed they have shown great potential for Point-of-Care applications in areas such as sample preparation, analyte detection, nucleic acid amplification (e.g. PCR) [7, 8] and cell analysis[9, 10]. One area where the centrifugal paradigm is particularly applicable is in pre-conditioning of whole blood. The centrifugal nature of these systems lends itself in an ideal manner to adoption of existing bench-top centrifugation based protocols such as density gradient centrifugation (DGC) (e.g. with Ficoll® medium) to isolate PBMCs from whole blood [11-15]. As expected by the theory of the Boycott effect, it was recently found that the sedimentation rate increases with the inclination angle or the channel with respect to the radial direction as well as the dilution level of the blood [13].

In this work we compare linear sedimentation chambers which have been inclined at 0°, 15°, 30°, 45°, and 60° (measured at the point closest to the centre of rotation) with chambers which closely approximate a Spira Mirabilis at the same pitches (Fig. 1b, c).

MANUFACTURING AND METHODS
The microfluidic disc was fabricated from 3 layers of PMMA bonded together using two of layers of pressure sensitive adhesive (PSA). Loading and centrifugation chambers are created by removing material from the central layers of PSA and PMMA. Microchannels connecting the loading and centrifugation chambers were created by removing material from the upper PSA layer.

Two disk configurations are tested. The first configuration, as shown in Figures 1a and 2, contains 5 linear chambers 1.5mm wide, 1.5mm deep and 22.5mm high. The chambers are angled relative to the radial centrifugal force (RCF) at 0°, 15°, 30°, 45°, and 60° (measured at the point closest to the centre of rotation).

Due to the length of the chambers and their close proximity to the centre of rotation, the angle of inclination relative to the centre of rotation decreases with the spacing from the centre of rotation. For example the 60° chamber has an angle of inclination of only ~30° at the point furthest from the centre of rotation, thus attenuating the Boycott effect towards the outer end of the chamber.

The second configuration, as shown in Figures 1b and 3, contains 5 curved chambers which closely approximate the design paradigm of the Spira Mirabilis at pitches of 0°, 15°, 30°, 45°, and 60°. Arc segments are chosen such that the sedimentation chambers contain the same volume of fluid. Measured from the point of the chambers closest to the centre of rotation, all chambers are on the same radius. In both configurations the mean distance from the centre of rotation of each chamber is less for the inclined chambers. Similarly the mean distance from the centre of rotation of the curved chambers is less than for the equivalent straight chamber. Thus these chambers are subjected to a lower RCF.

The chambers were tested with whole blood overlaid on the density gradient media (Ficoll®). Initially 32 µl of Ficoll® 1077 Density Gradient Medium, is pre-loaded into each chamber. The disk is briefly centrifuged to ensure the Ficoll® is settled in the bottom of the chambers (Fig. 2a and Fig. 3a). 20 µl of whole blood (1:1 dilution) is then overlaid on the Ficoll media and the disks are centrifuged. Centrifugation is conducted at 60 Hz to generate an RCF of ~400 g at the base of the vertical (0°) chamber. This RCF is typical of bench-top protocols for DGC.

Previous studies of the Boycott Effect on lab-on-a-disc platforms [13] have tracked the position of the hematocrit height to determine the progress and identify the completion of processing of whole blood. However, in
the case of DGC, the close density match between erythrocyte and the density medium means a clearly defined hematocrit cannot be located. Therefore the progress of sedimentation is measured through monitoring the intensity of a region of interest (ROI) in each chamber.

The ROIs were defined above the final hematocrit aggregations and below the PBMC layer. The mean image intensity in the ROI was measured in each image. The erythrocytes were deemed fully sedimented when the normalized intensity returned to 80% of the maximum intensity measured in the plateau. Figures 4a and 4b show the typical normalized measurements of image intensity taken during experiments.
RESULTS AND DISCUSSION

Figure 4c shows the times taken for the blood to fully stratify in the density gradient media in each chamber tested. The 0° chambers for both straight and spiraled configurations are in effect identical. These chambers take approximately two minutes for stratification to complete. Increasing the angle of inclination/pitch of the spirals from 0° to 60° results in a shorter sedimentation time at each intermediate step. Along with the enhanced performance of angling the chambers compared to the straight chambers, at every non-zero angle of inclination the sedimentation time in the spiral chambers is faster than in the linear chambers. This effect is present despite the spiraled chambers being subjected to a lower mean RCF due to their closer proximity to the centre of rotation.

The chambers at a 45°incline/pitch showed the greatest improvement where the sedimentation in the spiraled chamber completed 25% faster than in the linear chamber. Compared to the non-inclined chambers (which took an average of 2 minutes to sediment), the spiraled chambers at 45° and 60° took just approximately one minute (a 49% and 53% improvement, respectively). However, consistent to work reported previously [12], it appears increasing the pitch/angle beyond a certain point offers limited reduction in sedimentation time.
CONCLUSIONS
Modeling our structures on the Spira Mirabilis significantly increases the rate of sedimentation in the chambers by up to 25% (for the 45° chamber) compared to conventional linear chambers. This is despite the curved nature of the spiraled channels resulting in these chambers being subjected to a lower RCF compared with the equivalent straight chambers. The equiangular property of the Spira Mirabilis means the Boycott effect is applied uniformly along the lengths of the chambers while in the linear chambers the angle of incline decreases in the radial direction. This is particularly effective in the case of density gradient media which typically occupy the lower portion of a sedimentation chamber. In addition, the close density match between erythrocytes and the density gradient medium compared with erythrocytes and plasma results in longer sedimentation times compared to processing whole blood.

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REFERENCES
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