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An unexpected phenomenon of RBC bouncing back and forth in a laminar flow channel.
An unexpected phenomenon of red blood cell bouncing back and forth between the walls inside a microfluidic channel was observed during experiments, and is presented as “Cell Pinball” in this paper. In general, cells in a microfluidic environment are supposed to move along the streamlines parallel to channel walls when the Reynolds number is small, and the inertia of the cells becomes negligible. However, the cell pinball presented in this paper does not only move along the streamlines but also move across the channel with velocity component perpendicular to the streamlines while the Reynolds number is only 0.74. Furthermore, the motion in the direction perpendicular to the streamlines reverses when the cell pinball hits a wall as it “bounces” at the wall. The phenomenon caught our attention, and is investigated with both microbead visualization and confocal microscope. Consistent patterns of rotation with respect to the directions of motion are observed. A kinematic model is proposed to interpret the phenomenon, and it is believed that the phenomenon is caused by the separation of the centroid of cell and the contact point. The model successfully interprets the features of cell pinball, and the estimated separation between the centroid and the contact point are presented.

1 What is Cell Pinball?

Cell pinball is a phenomenon of cells moving like pinballs inside a microfluidic channel while the cell inertia is negligible due to scale effect. In other words, such bouncing motion is not supposed to happen, and the pinball-like motion should be a result governed by a mechanism inside the microfluidic channel other than the inertial effect of cells. This paper is focused on realizing the mechanism behind the phenomenon.

Fig. 1 shows examples of the red blood cells (RBCs) in the microfluidic channel from the experimental results. In Fig. 1(a), RBCs are shown in motion blur due to fast flow which is driven by a significant pressure drop inside the channel. The direction of RBCs motion can be found moving along streamlines parallel to the channel wall, and the motion is as expected because of the laminar flow in fluidic system with a small Reynolds number. Fig. 1(b) shows RBCs flowing with hypotonic solution in the same microfluidic channel. Although some RBCs remain rapidly moving along the streamline, other RBCs move at much slower speed, about one percent of the fast ones, and “bounce” between the channel walls as indicated by blue and red arrows in Fig. 1(b).

Fig. 2 shows another type of RBC motion whose speed is lowered as the bouncing ones in Fig. 1(b), but it doesn’t “bounce”. Three observed facts from Figs. 1 and 2 are summarized as: (1) cell pinball moves slower than surrounding fluid. (2) Pinball-like motion was unexpectedly observed during experiments.

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**Fig. 1** Snapshots of RBCs inside a microfluidic channel. (a) Generally, cells are flowing along the streamline in the channel. (b) Pinball-like motion was unexpectedly observed during experiments.
laminar flow environment. (3) Some cells don’t move across the channel nor bounce, but they move at a lowered speed as cell pinballs. These examples show that RBC behavior significantly changes under different solutions, and may affect cell operations in a microfluidic channel.\(^2\)\(^-\)\(^6\) To assure such pinball-like behavior not interfere with on-chip cell manipulation, the reasoning of the behavior is investigated and interpreted. Microbeads and confocal microscope are employed for studying the phenomenon, and we found the cause of the pinball-like motion is very likely due to the rotational motion of each cell pinball. Consistent patterns of rotation with the direction of bouncing are found, and are interpreted by the proposed kinematic model. Part of the results in this paper has been previously presented in the form of a conference paper,\(^7\) and a more comprehensive analysis and discussion are additionally added to this paper.

The rest of this paper is organized as follows. Experimental conditions for generating cell pinball and cell pinball motion and shape obtained through visualization methods are presented in Section 2. According to the observed fact from experiments, a mechanism for cell pinball phenomenon is proposed in Section 3. The features of pinball phenomenon are discussed with the proposed model in Section 4 before concluding this paper in Section 5.

## 2 Experiments

This section includes two parts of experiments. The first half is focused on the conditions for generating cell pinball which covers the fabrication of the microfluidic channel and the concentration of sodium chloride NaCl in the suspension fluid driving the cell flow. The second part is on the visualization of cell movement by attaching microbeads and using confocal microscope. The visualization provides informative insights for the reasoning of cell pinball phenomenon.

### 2.1 Experimental System and Channel Fabrication

The experimental system includes a microfluidic channel which is fabricated inside a microfluidic chip, a syringe pump, a pressure sensor (COPAL ELECTRONICS: PA-830-102A-10) for measuring the pressure at the inlet of the microfluidic channel, a microscope (OLYMPUS: IX71) for observing the movement of RBCs in the microfluidic channel and a high-speed camera (Photron: FASTCAM MH4-10K) for recording the movement of RBCs. The flow inside the microfluidic channel is driven by a constant pressure difference between the inlet and outlet of the channel, and the pressure difference is controlled by a feedback control system with the pressure sensor and the syringe pump.

Fig. 3 shows a scheme of the microfluidic channel. The height and width of the channel are 4[µm] and 50[µm], respectively. The Reynolds number in the microfluidic channel is calculated as \(Re = \frac{\nu L}{\eta}\) with the assumption of the fluid viscosity equal to \(8.93 \times 10^5 [\mu m^2/s]\). Since typical biconcave RBC thickness varied from 600 [nm] at the central region to around 1.2 [µm] at the edge,\(^5\) RBCs are not supposed to constantly having contact with the channel walls in such a 4[µm]-high microfluidic channel.

The microfluidic chip is made by molding the channel pattern from a master mold with polydimethylsiloxane (PDMS), and the mold is made by standard laser lithography.\(^9\),\(^10\) SU8-3005 is used for negative photoresist and the mixing ratio between PDMS and curing agent is 9:1.

### 2.2 Osmotic condition – Concentration of NaCl

Cell pinballs are discovered when cell flowing in hypotonic saline solution (NaCl \(< 0.9\%\)) as explained in Section 1. It is well known that RBCs are inflated in hypotonic solution as additional water flows through the cell membrane into the RBCs.\(^1\)\(^1\) This gives a clue of the phenomenon that cell pinball has something to do with RBC shape. RBCs may be inflated so much that started to have contact with the ceiling and floor of the channel, and such geometrical constraints are believed to be the cause of pinball-like motion.

For determining an appropriate osmotic condition for the experiment, the concentration of NaCl and the appearance of cell pinball is tested with RBCs from four volunteer subjects who have read and agreed the consent of the test. The RBCs are firstly placed in the solution with specified NaCl concentration for 5 minutes, and then are injected into the channel. The flow inside the channel is driven by a constant pressure drop between the inlet and outlet of the channel. The cell flowing inside the channel is captured by the high-speed camera at the frame rate of 500 frames per second (fps), and a total number of 200 RBCs were counted for each NaCl concentration. The results are shown in Fig. 4. There are no RBCs in cell pinball mode for the cases

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**Fig. 2** An example of Cell Pinball without bouncing between the channel walls.

**Fig. 3** A schematic diagram of a Cell Pinball moving in the microfluidic channel.
where NaCl concentration is more than 0.8% or less than 0.2%. It is believed that the RBCs are not inflated enough to make contact with the ceiling and floor of the channel for the concentration over 0.8%, and may be over inflated so the RBCs are already collapsed in the concentration less than 0.2%. Coincidentally, the appearance percentage of cell pinball reaches a peak when the concentration of NaCl is 0.5% for all four subjects. Thus, saline with NaCl concentration of 0.5% is used for the experiments.

2.3 Visualization - Microbeads Attachment

In order to observe how the RBCs in cell pinball mode move, we used microbeads for visualizing the motion, rotational motion in particular, of the RBCs in microfluidic channel. The microbeads (Carboxyl latex beads, 0.8µm, Invitrogen) are attached to the surface of the RBCs as markers. Fig. 5 shows two examples of sequenced photos of cell pinball and beads, which are highlighted by red circles. The sequenced photos are stitched together from multiple video frames which are selected based on the x position of the cell pinball. The horizontal distances between every two cells are specified as 10µm and 20µm for Fig. 5(a) and (b), respectively. In Fig. 5(a), we find that the RBC rotates clockwise while moving upward, and starts to rotate in counterclockwise direction after hitting the upper wall. A total of 23 cell pinballs are examined, and there is no exception in terms of the directions of rotation. This consistent rotation to the cell motion provides another clue to the pinball phenomenon.

Fig. 5(b) shows an example of slow-down RBCs, as the one in Fig. 2, with microbeads attachment. The RBC moves at a much lower speed than surrounding RBCs, but it does not rotate as the beads constantly located at the upper left side of the RBC. The lowered speed is believed due to the geometrical constraints from the ceiling and floor of the channel as the cell being inflated by hypotonic solution. In other words, additional resistance forces against cell moving forward with the flow is generated as a result of the contact between cell and the channel.

It is observed during the experiments that the amount of y displacement of the RBC is corresponding to the amount of rotation of the RBC. Fig. 6 shows a sequence of cell pinball images where the RBC is moving from lower-left corner to upper-right corner of the image. The trajectories in blue and orange curves represent the trajectories of the cell pinball and the attached microbead, respectively. The trajectories are calculated based on the initial and final positions of the RBC from the captured images and the amount of RBC rotation with the assumption of uniform translational and rotational motion. The assumption is to suggest that the motion across the microchannel, which is the cell motion along y axis, is solely due to the cell rotation. In other words, if a cell does not rotate, the cell would move along the stream lines without any motion on y direction according to the assumption. The actual microbeads trajectory perfectly matches to the calculated microbeads trajectory, and it means the translational and rotational motion of the RBC are associated. In other words, the motion of RBCs along y direction in cell pinball mode is possibility caused by RBCs rotation.

2.4 3D Visualization - Confocal Microscope

For directly observing the cell shape inside the channel, the RBCs are placed inside the channel filled with phosphate buffered saline (PBS) solution with 0.5% of NaCl, and is then scanned by a confocal laser scanning microscope (OLYMPUS: FV-1000D) for constructing its 3D configuration. The RBCs are stained with the fluorescent dyes (Calcein-AM solution, Dojindo Corp.) advance for the confocal microscopy. Fig. 7 shows the result of the 3D observation of the RBCs from the confocal microscope with different view fields and light settings. Since only RBCs were stained by the fluorescent dyes, the walls, ceiling and floor of the microfluidic channel are not visible but only the RBCs. According to Fig. 7, we can see that the upper and lower surfaces of the RBCs are flattened, and these flattened surfaces are believed rep-

Fig. 4 The relationship between the concentration of NaCl and the chance of seeing cell pinball in a flowing microfluidic channel.

Fig. 5 Examples of cell rotation with microbeads visualization. (a) The rotational motion of cell pinball is observed with microbeads indication. (b) Cell doesn’t rotate when it moves along the flow.
Fig. 6 The comparison between the experimental data and expected trajectories.

Fig. 7 A direct observation of 3D cell shape inside the microfluidic channel.

Fig. 8 Possibilities of cell postures inside the microfluidic channel in the experiments.

Fig. 9 The kinematic model for explaining Cell Pinball.

3 Cell Pinball Mechanism

Two clues of cell pinball phenomenon have been discussed in the previous sections, and they are (1) cell pinball only happens in hypotonic solution. (2) cell pinball rotates clockwise when moving upward and counterclockwise when moving downward. According to these clues, it is believed that cell pinball is directly related to the shape of RBCs and the contact to the ceiling and floor of the channel.

3.1 Possible Shape Configurations

Fig. 8 shows the side view of possible RBC shapes under different conditions. The gray boundaries represent the ceiling and floor of a microfluidic channel. Fig. 8(a) shows RBC shape in isotonic saline solution (\(NaCl = 0.9\%\)) while Figs. 8(b-1) and (b-2) show two expected shapes of RBC in hypotonic solution (\(NaCl < 0.9\%\)). The difference in the two expected shape in (b-1) and (b-2) are the amount of inflation. The one in Fig. 8(b-1) is slightly inflated by osmotic condition, and only the edge of the biconcave ring touches the ceiling and floor of the channel. The one in Fig. 8(b-2) is becoming a sphere due to full inflation, and the contact point, or area, lies on the center of the cell. The solid lines indicate the lines passing through the mass center of the cells along z-direction. Figs. 8(c-1) to (c-4) show the cell deformation while under a fluid flow from the left to the right. The cells are additionally deformed in horizontal directions by shear stress from the flow, and the dashed lines indicate the lines passing through the contact points to the ceiling and floor of the channel along z-direction. We would like to specially emphasize the separation between the lines passing through the mass centers, the solid lines, and the ones passing through the contact points, the dashed lines. The separation of the solid lines and the dashed lines for each RBC model results in a nonzero moment for cell rotation, and that is believed to be the reason of cell rotation as observed in Fig. 5. For the convenience of reading, the lines that passing through mass centers and contact points are referred as centroid line and contact line in this paper, respectively.

3.2 Kinematic Model

According to the possible deformations shown in Fig. 8 and the discussion on them, a kinematic model based on the separation of centroid line and contact line is proposed. Fig. 9(a) and Fig. 9(b) illustrate the model from the top view and the bird’s-eye view, respectively.\(^{17,18}\)

The rotation of cell pinballs can be explained by the model previously shown in Fig. 8. We assume the contact line to be the rotation axis if resultant moment on the cell is not zero. In Figs. 8(c-1)
and (c-3), the contact line is located on the downstream side of the centroid line. While the cell is under the shear stress from the flow, such a configuration is very unstable. A slight shift between the centroid line and the contact line would result in a nonzero moment to rotate the cell with respect to the contact line. For example, if the centroid line slightly shift higher than the contact line from a top view as in Fig. 9(a), a moment in clockwise direction is then generated by the flow and the y displacement between the centroid line and the contact line. Once the cell rotates in clockwise direction, the y displacement increases. As a result, the moment diverges and becomes greater and greater. The rotation of cell pinball is also confirmed on a commercial finite element analysis (FEA) software COMSOL, and the details can be found in the appendix in ESI.

On the other hand for the case of Figs. 8(c-2)(c-4), the contact line is located at the upstream side of the centroid line, and this configuration makes the RBC stable regarding rotational motion. It is because when a displacement along y direction between the centroid line and contact line, the direction of the moment always try to align these two lines in the way that minimize the displacement in y direction. Therefore, a cell in Figs. 8(c-2)(c-4) configuration is expected to not rotate even if it has contact with the channel.

4 Discussion

According to the proposed kinematic model of cell pinball mechanism in Section 3, the features of cell pinball phenomenon are discussed in this section.

4.1 Why not moving along stream lines?

Fig. 10 illustrates the proposed mechanism where the white and black dots represent the contact line and the centroid line, respectively. The RBC in cell pinball mode moves along y direction because of the rotational motion in the channel. The RBC starts to rotate counterclockwise with respect to the contact line from an unstable state as in Figs. 8(c-1) or (c-3). When the RBC rotates, instead of moving along the stream line the RBC moves to the lower right direction as shown in Fig. 10(b). At the same time, the direction of the force applied to the RBC from the flow changes due to the rotation, so that the configuration of RBC shape changes which results in a shift of contact point as shown in Fig. 10(c). Assuming the sequence from Fig. 10(a) to Fig. 10(c) repeats, the cell pinball then moves across the channel with a counterclockwise rotation as the rotation observed in Fig. 5.‡

4.2 Why Cell Pinballs bounce at channel wall?

Fig. 11 illustrates the proposed bouncing mechanism where the white and black dots represent the contact line and the centroid line. Assuming a cell pinball is rotating counterclockwise and moving toward lower-right direction as shown in Fig. 11(a). When the cell makes contact with the wall, as in Fig. 11(b), the rotation stops due to a significant amount of friction applied to the cell from the contact as well as the geometrical constraint preventing the cell continues to move downward. Hence, the cell can either move upward with clockwise rotation if the cell configuration is like Figs. 8(c-1) or (c-3), or it can move along the streamline if the cell configuration becomes stable as Figs. 8(c-2) or (c-4). In all cases in the experiments, cell pinballs end up moving upward because the chance of hitting the wall and change to a stable posture is relatively small. Therefore, cell pinball moving back and force between channel walls just like an elastic object bouncing between two surfaces.

4.3 Why some cell having velocity dropped but no bounce?

Fig. 12 illustrates the mechanism of the cell having their velocities slow-down but remains moving along the streamline. These cells are previously mentioned as in Fig. 2 and Fig. 5(b). The white and black dots in Fig. 12 represent the contact line and the centroid line, respectively. Fig. 12(a) shows that the centroid line is located on the downstream side of the contact line, which makes the cell stable in rotation as in the configuration of Figs. 8(c-2) or (c-4). Even a cell starts from a configuration that the centroid line and the contact line are not horizontally aligned as shown in Fig. 12(b), the direction of the moment due to the flow would rotates the cell, so that these two lines are aligned in x direction as shown in Fig. 12(c).

4.4 Correlation between Cell Size and Pinball Motion

Fig. 13 shows the geometrical relations between cell pinball movement, centroid line and contact line. Assuming the pinball

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‡ The motion of cell pinball can also be found in the video clip in ESI.

† The configuration is similar to a classical system of inverse pendulum where the pendulum falls if its mass center and the connecting joint are not on the same gravitational line that is perpendicular to the ground.
motion along $y$ direction is only caused by the rotational motion, the distance between the centroid line and contact line can be derived as:

$$S = \frac{L}{2\pi n}$$

where $L$, $n$ and $S$ are the displacement of the RBC along $y$ direction, the amount of rotation in revolution, and the displacement between the centroid line and the contact line, respectively. An example of computing $S$ from an experimental result where parameters were measured as $L = 38.64[\mu m]$, $n = 5.0[rev]$ and the radius of the RBC $R = 3.59[\mu m]$. According to Eq.(1), $S = 1.22[\mu m]$ can be obtained.

Fig. 14 shows the relationship between the diameter of the RBCs in cell pinball mode and the values of calculated $S$ based on their trajectories and amount of rotations $n$. The values of $S$ are consistently smaller than corresponding cell radius, $D/2$, which indicates that $S$ obtained from the kinematic model is within a reasonable range from the perspective of geometry. The values of $S$ are also positively correlated pinball diameter with $R^2 = 0.6675$ in a linear fit. It shows that a larger cell pinball usually with a greater displacement between its centroid line and contact line. The separation distance $S$ contains the information of RBC deformability due to both deformation by the geometrical constraint and fluid flow, and could be utilized as a new index for cell evaluation.

5 Conclusions

In this paper, we observed an unexpected phenomenon happening inside a microfluidic channel, and named it as “cell pinball.”

§ The details of derivation can be found in the appendix in ESI.

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References

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