Simulation and experimental research on micro-channel for detecting cell status in bio-artificial liver

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Abstract.
BACKGROUND: Bioartificial liver support system (BALSS) based on culturing hepatocytes is an important research field for the treatment of acute liver failure. It is necessary to monitor the state of liver cell functions during the treatment of BALSS in order to guide clinical treatment.
OBJECTIVE: To design a micro-channel chip to achieve flash mixing for timely detection of liver cell status in bioreactors and improving liver cells growth environment to ensure the efficacy of the bio-artificial liver support system.
METHODS: Alanine aminotransferase (ALT) and Urea are chosen as detection indicators to reflect the degree of liver cell injury and the detoxification function. A diamond tandem structure micro-channel is designed and optimized to achieve the efficient mixing of serum and ALT or Urea reagent.
RESULTS: The simulation and experimental results show that the diamond tandem structure micro-channel can significantly improve the mixing efficiency and meet the online detecting requirements.
CONCLUSION: The easily controllable diamond tandem structure micro-channel combines the advantages of active and passive mixer and can effectively mix the serum and ALT or Urea reagent. It lays the foundation for online monitoring of liver cells and will help to improve the viability of liver cell in the bioreactor.

Keywords: Bio-artificial liver, ALT, urea, ANSYS, mixing efficiency

1. Introduction

Liver failure is a disease with a mortality rate of up to 80%. Bio-artificial livers are used in the treatment of patients who suffer from liver failures. The core of a bio-artificial liver is the bioreactor, where liver cells grow and function. Therefore, the microenvironment in bioreactor is directly related to the efficacy of the bio-artificial liver. Currently, the detection of the status of liver cells in bioreactors is conducted offline and the operations are cumbersome, and the feedback of which cannot be directly used to adjust the microenvironment in a timely manner. In this paper, we designed an innovative micro-channel, which can help evaluate the liver cell status in real time. Based on the principle of optical colorimetry, Alanine aminotransferase (ALT) and Urea were chosen as detection indicators to reflect the degree of

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liver cell injury and the detoxification function. A spectrometer was used to detect the change of absorbance at wavelength of 340 nm caused by the concentration of ALT or Urea [1]. The concentration of ALT and Urea could accurately reflect liver cell growth and functioning status, and therefore are important indicators for bio-artificial livers to improve efficacy.

Since serum transfusion often exposes the liver cells to infection, in long-term cell status monitoring, it is necessary to minimize the usage of micro-channel which consumes serum in the detections This micro-channel needs to achieve efficient mixed reaction to ensure the reliability of online detecting. Common micro-mixing methods can be classified into two categories: active mixing and passive mixing. Active mixing requires external energy, such as magnetic, acoustic field force or electric force [2]. Bruyker et al. [3] designed an active mixing based on magnetic actuation using micro machined magnetic stir bars last year. Ryu et al. [4] designed a micro magnetic stir-bar integrated in parylene. Although both of them have high efficiency and small mixed-scale, they need complex processing and integration, which made them difficult to be widely applicable. Due to its simplicity and easy fabrication, passive mixing is an more attractive solution for most cases. Common types of passive mixers include: T/Y type micro-mixer [5,6], wavy channel micro-mixer, micro-mixer with static resistance, and so on. Sui [7] simulated a wavy channel micro-mixer to enhance mixing by increasing Reynolds numbers. Naher [8] and Xu [9] optimized mixing effect by comparing different kinds of obstacle structures. Jeon [10] designed a zigzag type micro-mixer, and proved that its mixing effect is better than the micro-mixer with static obstacle. These structures still cannot meet our requirements of mixing. Although their structures are different, their principles are the same to improve mixing effect by changing geometric structure to increase turbulence or to reduce the diffusion distance.

In the present study, we designed a diamond tandem structure micro-channel which can repeat the cycle of split-and-recombine to achieve better mixing. Simulation software ANSYS14.0-Fluent was used to optimize the structure [11]. Experiments were carried out to verify the effectiveness of the micro-channel.

2. Theory and modeling

The structure of diamond tandem micro-channel is shown in Fig. 1. The length of the model is 4 cm. Section 1 represents the reagent inlet and Section 2 represents the serum inlet. In order to facilitate the matching of the inlet pipe diameter, the inlets of the micro channel width is set to 800 μm. The width of the subsequent inlets were decreased progressively to 500 μm, 300 μm, 200 μm, 150 μm and finally to the standard width of 100 μm. The ratio of the width of the micro-channel to the depth is generally less than 5:1 in order to avoid the deformation and depression. Based on this, the micro-channel depth was set to 200 μm. We used hexahedral grid to mesh the micro-channel and set the boundary layer grid. The maximum size of grids was 10 μm and the total number of grids was more than 3 million.

The designing idea is shown in Fig. 2. Serum is represented by the color red and ALT or Urea detection reagent is represented by the color blue. The liquid from the straight-shaped channel flows into the rhombus-shaped channel and was divided into two branches. The angle between the two branches is 90°. As shown in Fig. 2, the flow velocity V can be decomposed into V_x and V_y. The direction of the flow velocity V_y is consistent with the direction of two liquids' concentration gradient, which can speed diffusion efficiency of two liquids in the direction of y [12]. The liquid flows into straight-shaped channel after outflows from the rhombus-shaped channel, the flow velocity V can also be decomposed into V_x and V_y. The direction of the flow velocity V_y is consistent with the direction of two liquids' concentration gradient, which can speed diffusion efficiency of two liquids in the direction of y too.
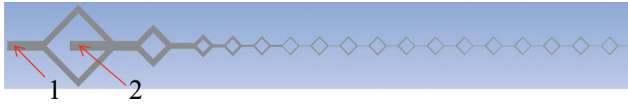
Fig. 1. Schematic diagram of the diamond tandem micro-channel.
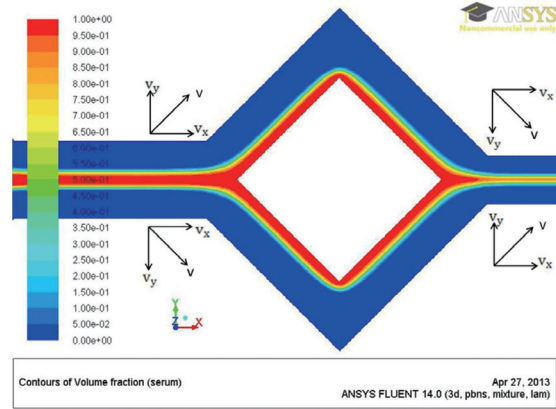

Fig. 2. Schematic of design idea.

More model elements in series can make the liquid constantly separate and merge, thus creating more opportunities for the concentration gradient and flow velocity V_y in the same direction to improve the mixing efficiency. Based on this principle, we can largely improve the mixing efficiency by increasing the number of the model element.

The length of the simulation model is 4 cm, as shown in Fig. 1. The commercial software ANSYS14.0-Fluent is employed in the numerical simulation. Computer simulation to Inter CORE i5 dual-core processor, 16 G memory, computation time is different for each model, about 3 days–4 days. The three dimensional Navier-Stokes equations were solved. The liquids need to be mixed in micro-channel are detection reagent and serum. Serum density is $\rho_{serum} = 1042.25$ kg/m^3 and viscosity is $\mu_{serum} = 4.15 \times 10^{-3}$ kg/m · s. The density, viscosity and other characteristics of ALT and Urea detection reagent are close to those of water. We use water to replace two kinds of reagent in the simulation. Reagent density is $\rho_{reagent} = 998.2$ kg/m^3 and viscosity is $\mu_{reagent} = 1.003 \times 10^{-3}$ kg/m · s. Serum and detection reagent are assumed to be newtonian fluid. The micro-channel characteristic scale is the micron level and the reynolds number is far less than 2000. Laminar model and multiphase mixture model are used [13]. The velocity-inlet and pressure-outlet boundaries are used. The flow rate of Vserum is 2.5 μl/min, VALT reagent is 25 μl/min and VUrea reagent is 250 μl/min.

Mixing efficiency α [14] and minimum mixing ratio η were chosen to reflect the degree of mixing. Mixing efficiency α is defined as:

$$\alpha = \left(1 - \frac{\sigma}{x}\right) \times 100\% \tag{1}$$

Where x is the ideal value of the mixing serum. Due to the flow ratio of 10 : 1 between ALT reagent and serum volume, the ideal value x is 9.1% when detecting ALT. When detecting Urea, the serum ideal mixing value x is 0.99%, due to Urea reagent and serum volume flow ratio is 100 : 1. Where σ is standard deviation.

$$\sigma = \sqrt{\frac{\sum\limits_{i=1}^{n} (c_i - x)^2}{n}} \tag{2}$$

Where c_i is serum volume distribution value in every compute node and n is the total number of all nodes in the computational domain.
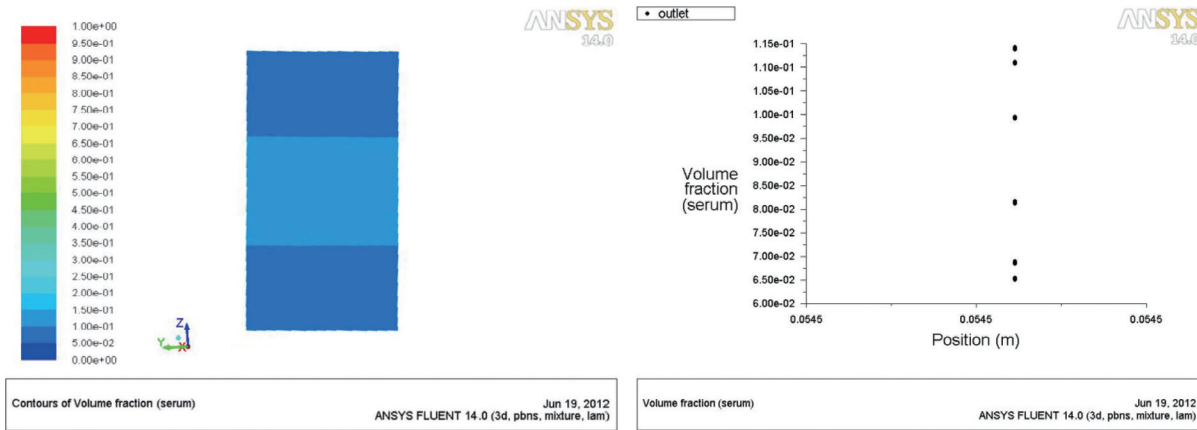
Fig. 3. Serum volume fraction contours of outlet.

Minimum mixing ratio η is defined as:

$$\eta = \min \left\{ \left(1 - \frac{\sqrt{(x - c_i)^2}}{x} \right) \times 100\% \right\}, \quad i \in [1, n] \tag{3}$$

3. Simulation and analysis

The mixing effect of ALT reagent and serum using the simulation model were as shown in Fig. 1, the flow rate V of the serum (Vserum) was 2.5 μl/min and the flow rate of ALT (VALT) reagent was 25 μl/min. The velocity-inlet and pressure-outlet boundaries were used. The wall was adiabatic with zero heat flux and the boundary was no-slip. Steady solver was used. After entire computational domain was initialized to be filled with serum, simulation was started. As shown in Fig. 3, the serum volume fraction of outlet range was from 6% to 11.5%. Mixing efficiency α is 78.9% and minimum mixing ratio η was 66%, which did not achieve complete mixture. The model needed to be improved.

As shown in Fig. 3, the serum was mainly located in the upper and lower hierarchical directions, and was unevenly distributed in the outlet. Therefore, we changed the model vertical structure to optimize the mixing effect. Comparisons of established models are shown in Fig. 4.

Comparing the four models in Fig. 4, we can discover the main difference with the original diamond tandem structure channel lies in vertical structure. In model A, the height of the straight-shaped channel was reduced to 100 μm. In model B, the height of the rhombus-shaped channel was reduced to 100 μm. Model C reduced all the height of the original diamond tandem structure channels to 100 μm. Model D reduced the height of the original diamond tandem structure channel to 100 μm and progressively reduced the width to 500 μm, 300 μm, 200 μm, 150 μm and 100 μm, respectively. According to the results of the simulation, the mixing effect of model C is best. The outlet serum volume fraction of model C ranged from 8.7% to 9.3%. Mixing efficiency α was 97.69% and the minimum mixing ratio η was 95.6%, which met the requirements of ALT online detecting. Model A, B and D failed to meet the threshold of the 95% mixing efficiency α and the 95% minimum mixing ratio η. The micro-channel chip was able to process in real time. Therefore, model C was chosen as the optimized micro-channel.
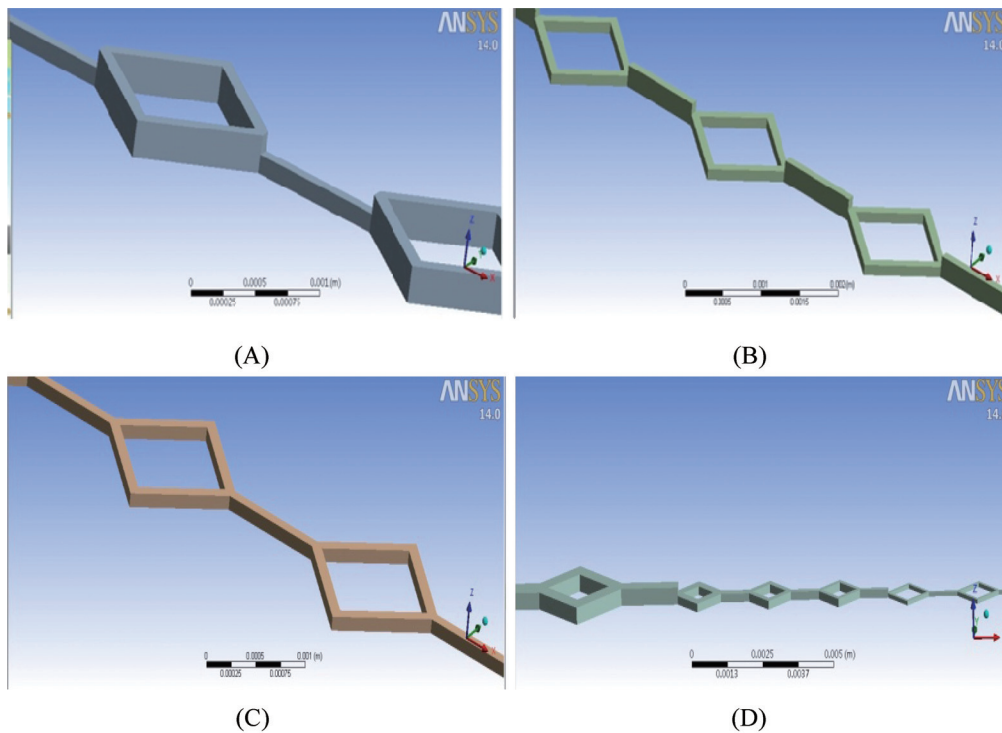
(A)

(B)

(C)

(D)

Fig. 4. Optimal comparison models.

The mixing effect of Urea reagent and serum was investigated using the model C, the flow rate Vserum was 2.5 μl/min and VUrea reagent was 250 μl/min. As the flow rate increased about 10 times, the mixing effect was not satisfactory. So we extended length of model C to 12 cm to improve mixing effect. The outlet serum volume fraction ranged from 0.997% to 1.00%. Mixing efficiency α was 99.97% and minimum mixing ratio η was 98.9%, which meet requirements of Urea online detecting.

4. Experiment and discussion

Based on the results of the simulation, we designed the diamond tandem structure micro-channel chip and detection platform for the detection of cell status in bio-artificial livers. The design is shown in Fig. 5. The diamond tandem structure micro-channel chip was made of polydimethylsiloxane (PDMS) [4]. Diluted distributor, deuterium light source and the spectrometer were used for online detection with the diamond tandem structure micro-channel chip together.

To verify the detection accuracy of the micro-channel chip, 6 serum samples containing different concentrations of ALT or Urea were used.

The detection platform in Fig. 5, detected six different concentrations of ALT and Urea. The experimental results met the detection accuracy requirement of ALT and Urea. Linear fitting diagram of the experimental results are shown in Fig. 6.

Experimental results show that the detected values of the diamond tandem structure micro-channel chip were consistent with the reagents reference values. ALT linear correlation coefficient was 0.999787 and norm of residuals was 1.7541 U/L. Urea linear correlation coefficient was 0.999829 and norm of

Table 1
Serum samples

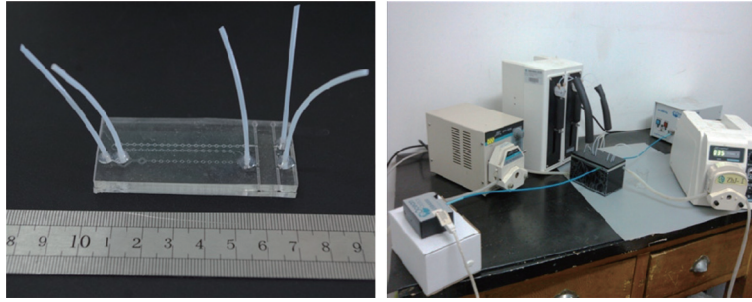| Serum samples	Concentration value of ALT (U/L)	Concentration value of urea (mmol/L)
Level 1	17.65	2.55
Level 2	35.3	5.1
Level 3	53.9	6.68
Level 4	72.5	8.26
Level 5	96.25	9.165
Level 6	120	10.07

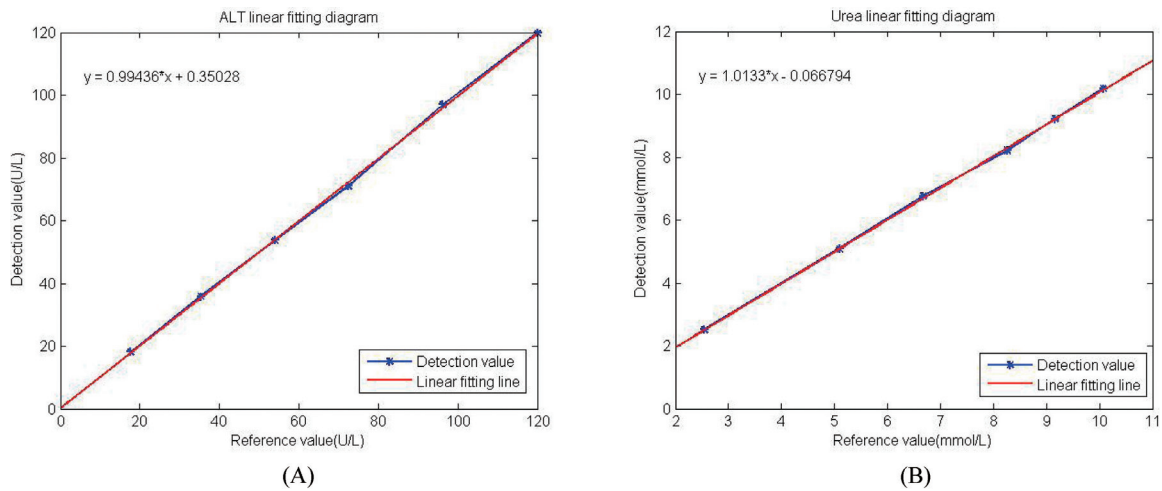Fig. 5. The designed and processed micro-channel chip and detection platform.

Fig. 6. Linear fitting diagram of the experimental results: (A) ALT (B) Urea.

residuals was 0.11728 mmol/L. All of the detected values had good stability, which could meet detecting requirements. The results also had a good linear correlation. The micro-mixer chip could deliver flash mixing and detection for bio-artificial livers.

5. Summary

The diamond tandem structure micro-channel was designed to accomplish better mixing effect for detecting cell status in bio-artificial liver. The simulation indicated our design yielded satisfactory mixing effects. The micro-channel chip can largely improve mixing effect without external force, which can easily be used in many biological and chemical fields compared to active mixers. The micro-channel chip is made of polydimethylsiloxane (PDMS). The total volume of the micro-channel chip was about 17 μL. When detecting alanine aminotransferase (ALT), the serum consumption was only 1.55 μL. When detecting Urea, the serum consumption is only 0.1717 μL. Due to the little serum consumption, this micro-channel chip can be used in real time online monitoring of the cell status without the need of frequent transfusion to the patients. Using the online micro-detection method for liver cell in bioreactor, we can provide a long time, continuous, low consumption and automatic detection for patients. This

method lays the foundation for optimizing the status of liver cell in bioreactor and is very important to improve the efficacy of the bio-artificial liver.

Acknowledgments

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