ABSTRACT

In this work, a microfluidic automatic concentration gradient generator (ACGG) for dissolved gas is demonstrated. The ACGG is formed by a serial of parallel micro-ladders cross linked by a smaller microchannel array. Surface energy is transferred to motivate the ACGG. During the capillary liquid filling process, the aggregation effect of the gas dissolved into the solution facilitated the gradient generation along the microladders. Based on this, both 1D and 2D stable CO₂ gradient has been generated. Moreover, chemical deposition of gradient Au nanoparticle is realized which is sensitive to CO₂ concentration.

KEYWORDS: Microfluidic, Gradient, Dissolved Gas, Au Nanoparticle

INTRODUCTION

In the past decades, microfluidics has made prodigious leap over delicate control of liquid niche environment. It has enabled fast development in application areas from biochemical and chemical process control. But in contrast to the prosperous liquid gradient generator, the research to dissolved gas gradient generator is limited.

There are mainly two categories of methods for on chip dissolved gas gradient generation. One category of methods for realizing dissolved gas gradient are based on gas in situ generation or reducing [1]. Scavenger or electrodes are integrated into the chip with proper distribution so as to realize spatial distribution of certain dissolved gases. Not only are these method largely limited by the range of selectable scavengers/or electrochemical reactions, but introduction of additional compartment would also complicate chip fabrication and lead to side effect to the process in the liquid. Another category of methods are based on the gas diffusion through conducting membranes, which usually holds slow speed for PDMS membrane mode [2] or compromised the chip transparency for faster diffusion speed by microporous structures [3]. Also, to generate gas gradient by diffusion, at least gas concentration of two on chip spots should be controlled. Moreover, both categories of methods require delicate control and/or additional peripheral equipment for gradient generation.

Actually, the microfluidic structure has inherited in itself the power to motivate the process and to polarize solution concentration [4, 5]. The capillary force, though negligible in macroscale, becomes one of the dominant forces when the structures approach microscale [6]. The capillary driven process in a hydrophilic microfluidic systems has been successfully manipulated in applications for process control. However, focuses are mainly on the dynamic filling ability for its application, its influence to the content of the solution is largely overlooked. In this work, we are to explore the capillary force induced concentration polarization, demonstrate with the model of ACGG for gas in this paper. Dissolved CO₂ gradient is taken as an example, which is visualized by the pH sensitive fluoresceine.

THEORY

The basic structure for ACGG is demonstrated in figure 1. The basic structure of the ACGG is composed of an array of microladders, cross linked by an array of narrower bridges. The microladder array is composed of 50 ladders 50 μm for width and 16 μm for depth and 1 mm for length, interspaced by 300 μm. The narrower microbridge array is composed of 12 channels 35 μm for width and 7.4 μm for depth and 15 mm for length, interspaced by 80 μm. Solution added in reservoir c will fill the super hydrophilic channel automatically due to the large capillary force in the system. The dissolved gas
concentration gradient (CG) would generate during the process and completely formed when the channel is totally filled with solution.

The principle behind the dissolved gas gradient is the adding effect of the constantly dissolved gas. It can be supposed if the dissolved gas concentration in one ladder is \( x \), since the fluid field is one directional, when the solution is to fill the next ladder, it would hold an original concentration of \( x \), and with the continuous gas dissolving, its concentration would increase a little to \( x + \Delta x \). As the liquid proceeds, the dissolved gas concentration will continuously increase until saturation in the front part of the liquid, which ultimately results in a dissolved gas CG.

However, it is found, in large microfluidic channels, CG generated is transient, which disappear quickly after the gas dissolving process is stopped. CG can be maintained if narrower bridging channel is introduced. This is because the reduced microchannel cross-section would help reduce the total flux induced by concentration gradient, although the strength of the flux is not changed.

EXPERIMENTAL

The PDMS chip is fabricated based on mold-copy. Air plasma treatment is applied to bond the PDMS chips and render the channel surface superhydrophilicity. The chip is used immediately after treatment.

To generate dissolved gas concentration, 5μL of 10mM fluoresceine solution is injected into the chip inlet. The source of CO2 comes from air. Waiting for the liquid to full fill the channels, which usually take 2~10 minutes, depending on the geometry of the channel. After the gradient is generated, the whole chip will be placed into a transparent box, which is saturated with water. In this environment, the gradient can be maintained for a long time and for fluorescence analysis.

To generate Au NP gradient, the solution is substituted by fresh mixture solution of 20% KHCO3, 1% HAuCl4 and 20% glucose (2:1:1 in volume), with all the other steps the same. The results are examined over 10 hrs with optical microscopy, AFM and SEM.

RESULTS AND DISCUSSION

The typical gradient in an array ladder structure is imaged in figure 2. The samples are collected from six equally distanced spots along the ladders. The fluorescence intensity is decreasing constantly from spot 0 to spot 5, indicating an increased acidity for the solution. To eliminate other possible factors (e.g. surface adsorption, size exclusion effect) that might induced fluorescence damping, pH insensitive rhodamin-6G and HAS-FITC is applied instead of fluorescein to character the gradient. The resulting homogeneity of the fluorescence intensity implied that the influence of other factors can be negligible in this system. Moreover, these results proved that a dissolved CO2 gradient has been successfully generated by the ACGG system.

![Figure 2. Phenomenon of ACGG in a microchannel array-ladders structure.](image)

![Figure 3. Stability analysis of the gradient](image)

To further exam the stability of the gradient, the fluorescence concentration in the last ladders is monitored once per hour. As is shown in figure3, the gradient can remain stable within 5 hrs, and even is acceptable over 18hrs in a sealed container. The sealed container here functions to prevent liquid evaporation, which might destroy the gradient by condensing the solution or inducing liquid flow in the system. The above result indicates the CG holds the potential for even long time applications.

ACGG in a 2D system is also designed and proved available based on the same adding effect of the dissolved gas. In this design shown in figure 4 left, the channel ladder array is transformed to a square channel matrix, while the narrow channel
bridges are transformed to cross linked structures. The generated gradient (figure 4 right) holds decreased fluorescence intensity from left to right and from top to bottom.

![Figure 4. 2D gas concentration gradient. Left: structure of the 2D ACGG chip, Right: fluorescence gradient.](image)

The 1D dissolved gas ACGG is further applied for Au NP deposition. Since the chemical deposition of Au NPs is sensitive to solution pH, this application can help us to both exam the quality of the pH gradient and explore the possibility of conducting chemical reactions in such system. The resulting deposited Au NPs are depicted in figure 5. It can be observed that slight pink color presents in the ladders (figure 5 left), which is the typical color of Au NPs. Transmitted light intensity analysis (figure 5 middle) show that the intensity of pink color decreases from ladder 1 to 5. A more close exam for the density of Au NPs can be get from the AFM and SEM images (figure 5 right) corresponding to each ladder. The largest density presents in the 1st ladder, which decreased quickly in ladder 2, 3, and 4, and is almost unobservable in ladder5. This result is in consistence with the Au deposition mechanism, where higher acidity will inhibit the process.

![Figure 5. Gradient for Au deposition](image)

**CONCLUSION**

In this paper, ACGG both for gas in 1D and 2D systems are realized by the hybrid structure of large microladders with the smaller microbridge arrays. The resulting gradient is proved stable and applicable for chemical reactions. It is expected to enrich the techniques for micro-environment control as for the concentration of dissolved gases.

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