# Microfluidic Devices for Stem Cell Study Fabrication Methods and Application

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## 1. Abstract

This review introduced the fabrication method and biomedical application of microfluidic device used in stem cell study. According to the context below, researchers used multiple approaches to culture stem cells and differentiate for subsequent study. In this paper, some pros and cons are also be illustrated and finally considering all the references mentioned, opinions of future promotion would be came up with as well.

**Key words**: Microfluidic device; Microfluidic cell culture; Stem cell culture; Stem cell differentiation; Organ-on-a-chip

## 2. Introduction

### 2.1 Microfluidic Device

Microfluidic device belongs to a type of micro device that finish some manipulation such as sample pretreatment, delivery, reaction, separation, detection and analysis. It has made great progress in the past ten years, due to its small fluid

volumes ( μ L,nL,pL), utilizing microfluidic device to bioanalysis can reduce device size. The unique advantages such as fast analysis speed, short response time, low amount of samples or waste and particular physical consequences of micro-scale fluid flow make it popular in biomedical field.

For manufacturing microfluidic devices, manufacturer can use photolithography, soft lithography or wet etching methods. In early stage, the microfluidic devices tend to use silicon or glass as the raw material. Manz's group firstly fabricated planar chips for integration and separation in 1992[1]. Meanwhile, Harrison et al. made a sample injection system[2]. At that time, authors often used photolithography approach until soft lithography method occurred which is mainly utilized polydimethylsiloxane (PDMS) as material. This terminology coined by Whitesides in 2001[3] .

PDMS is a kind of elastomeric materials. Microfluidic devices often used PDMS to fabricate microchannels. Silicon wafer with designed micropatterning was fabricated firstly, coating with photoresist and exposed to ultraviolet light subsequently. Afterwards, transparencies can be used as photomasks for quickly prototyping. In this process, PDMS mold can be replicated multiple times[4]. The photolithography process is easily repeated on the wafer to produce multiple layers of patterned photoresist and thin films of patterned PDMS can be stacked together to create a multilayer 3D microfluidic system which plays a overwhelming role in micromachining[5,6].

To date, microfluidic technology has been successfully applies to various biological applications including DNA or RNA sequencing determination, PCR

amplification, peptide or protein analysis, cell sorting and manipulation , etc. Among these applications, one is the most important is cell culture which is the basement of microfluidic device for stem cell (SC) study.

## 2.2 Stem Cells

Generally, SCs are originated from two main sources, adult body tissues and embryos. As a type of totipotent cell, it is available to differentiate any kind of cells and it proliferates indefinitely in vitro. Depending on different sources, researchers can extract and culture embryonic stem cells (ESCs) from embryos[7], however, these SCs extracting from adult body called adult stem cells. Compared to ESCs, these adult stem cells lose some abilities, which means they can only differentiate partly categories of cells. For example, hematopoietic stem cells (HSCs) produce blood and immune cells[8]; neural stem cells (NSCs) can differentiate different kinds of neurons[9], Mesenchymal stem cells (MSCs) can be differentiated into osteoblasts, adipocytes, and chondrocytes etc[10].

In 2006, Yamanaka discovered a method to induce adult cells back into stem cells which is called induced pluripotent stem cells (iPSCs)[11]. Subsequently, SCs therapy is proposed because SCs cultured from autologous mature cells have no immune rejection. Meanwhile, the use of SCs can treat some previously unachievable diseases, such as bone defect. By utilizing mesenchymal stem cells, doctors can realize bone regeneration[12]. It has been proved that MSCs have potential to treat damaged heart tissue, nerve system disease, vasulcar disease, osteogenesis, bone and cartilage

defect,etc[13]. HSCs have been used to improve the treatment of autoimmune disease[14]. Brain injury studies and central nervous system diseases have been reported treated by NSCs[15].

According these articles illustrated, it is undeniable that SCs can differentiate different kinds cells in vitro by adding specific inducting condition. However, the heterogeneity and sensitivity to specific environments of SCs and their mechanisms are not fully understand until now.

Traditional techniques have limited understanding of SC differentiation mechanism. In order to overcome the limitations of traditional SC research methods, microfluidic technology has become a powerful tool for SC analysis.

## 2.3 Cell Culture

When SCs is *in vivo*, they are in a 3D dynamic microenvironment. At that time, these cells can interact with surrounding cells or extracellular matrix (ECM) easily. They are exposed to low oxygen content and growth factor gradients which is very important to cell behaviors including differentiation, interaction, metabolism, apoptosis, self-renewal,etc. The interaction between cells and environment provides some signals for regulating cell responses. Different signals represent different cell responses. Considering this mechanism, it is difficult to simulate these different signals *in vitro* culture.

In traditional in vitro approaches, authors may use two-dimensional adhesion culture. At that time, it is difficult to simulate real biomechanics such as fluid flow

and shear stress in 2D environment[16]. Therefore, *in vitro* experiment often trigger unexpected differentiation and death rate is high. Compared to 2D environmental Petri dish, in microfluidic device, the microenvironment can be three-dimensional for a detailed understanding of the various interactions and signal transduction. Therefore using microfluidic device cultures SCs have tremendous potential. Authors can used it to proliferate, analyze and differentiate SCs.

## 3. Fabrication Methods

The fabrication process can be divided into microfluidic device manufacture and stem cell sample preparation. Retrospecting articles,several advanced microfluidic channel design have utilized in SC study[17-23].

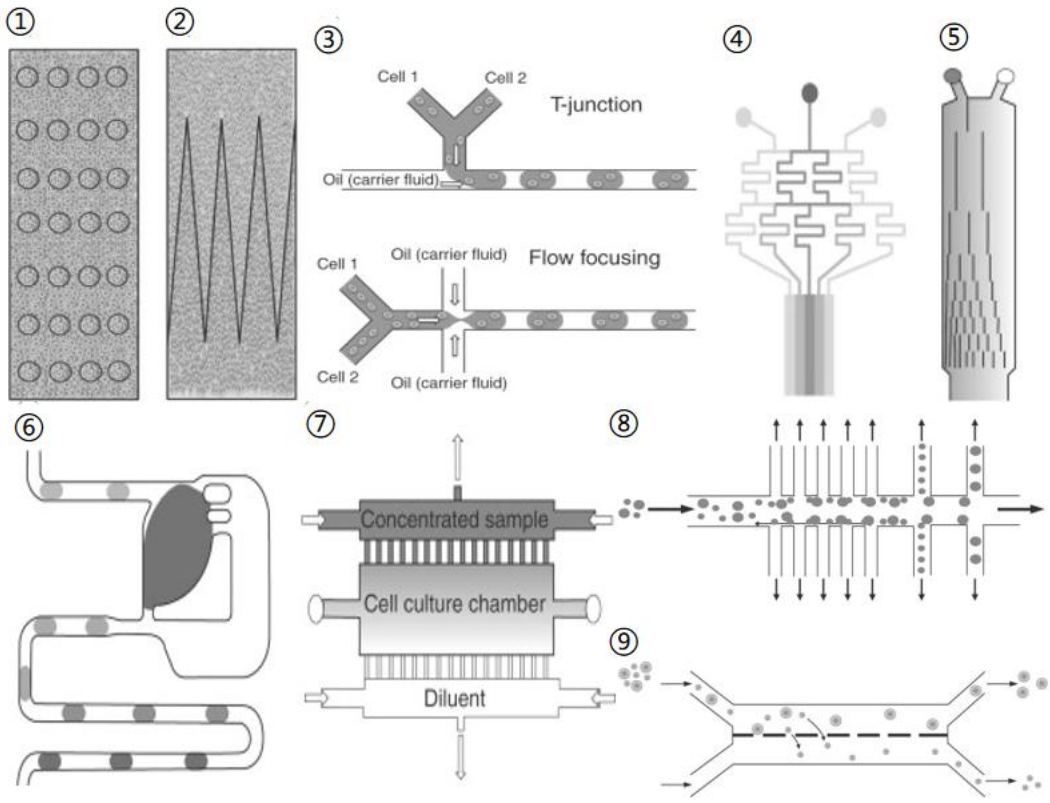


Figure 1. several advanced microfluidic techniques design[17-24]

Microfluidic platforms ①Micropores chip ②Silicon comb structure ③Microdroplet device; Concentration gradient generators ④Christmas tress structure ⑤Universal generator ⑥Droplet diluter

⑦Sink/source chamber; Cell sorting devices ⑧Hydrodynamic filtration ⑨Porous microfiltration

In Figure1, picture 1,2 and 3 are common microfluidic platforms in experiment. Authors tend to utilize this kind of microfluidic platforms to analyze various properties and behaviors of cells. For example, device in picture 1 is often used to study biophysical regulation of SCs[17]. Authors developed the micropores combined with multichannel so that it is easier to analyze cell properties. Device in picture 2 is often used to study the dynamics of intercellular communication between two kinds of cells[18]. Microdroplet device in picture 3 was developed to avoid contamination[19].

Concentration gradient generators in 4,5 was developed for simulating the biochemical microenvironment *in vivo*. It has been proved that a wide range of biological process including proliferation, migration, differentiation are determined by the gradient of specific molecular cues [20,23].

The microfluidic design as shown above often requires to build on a chip that often uses PDMS as material. Generally, the chip includes inlets, outlets and multiple microchannels. This part will combine with biomedical application and describe in the next section. In this section, detailed fabrication processes and SCs culture will be illustrated.

## 3.1 Fabrication Procedure

Cuchiara et al. proposed a multilayer microfluidic device to study cell migration. They used PDMS combined with polyethylene glycol (PEG) hydrogel microarchitecture which is very popular in the microfluidic device fabrication for SC

study[25]. Therefore, in this section, procedures proposed as an example also used this method.

Hydrogel commonly used in microfluidic device. It formed by exposing a mixture of PEG diacrylate (PEGDA) and photoinitiator to ultraviolet (UV) light. The microfluidic chip is simulated by the hydrodynamic model used for the diffusion process of biomolecules and the gradient generation. Liu et al. fabricated a PEG hydrogel covalently immobilized with RGD peptide gradient in the microfluidic device by photopolymerization[26].

**Fabrication of photoresist masters**

The first step is preparing photoresist masters.If organic or inorganic contaminations are present on the wafer surface, therefore cleaning procedure is required before coating photoresist on the glass slide.

SU-8 is a kind of negative photoresist. After glass slide was hrdroxylated and dehydrated respectively for 30 and 15 minutes, photoresist was coated and afterwards, transparency masks were used to align, expose UV light and develop. For negative photoresist, the part exposed in UV light would reserve on the glass slide.

In Cuchiara's experiment, in order to mold PDMS housing and PEGDA microchannels, two glass slides were used. PDMS housing can be molded by SU-8 2100. PEGDA microchannels can be molded by SU-8 2050. The former is thicker than the later. In Cuchiara's design, PDMS housing photoresist master (master1) is 350 μ m and PEGDA microchannels photoresist master (master2) is 100 μ m. Both of

the photoresist masters ought to cleaned with ethanol after finishing the development.

**Fabrication of PDMS mold**

PDMS were poured on master 1 to obtain the mold A. After the mold cures, remove PDMS housing and punch a hole into the port with a biopsy puncher, insert a PEG tube into the port and seal it with a compression fitting. Subsequently, PDMS were treated a serious processing for promoting free radicals induced interfacial polymerization of PEGDA.

After drying, PDMS mold 1 was put on the master 2. Because master 1 is thicker than master 2, there is a tiny gap between PDMS and photoresist master 2. PEG hydrogel was primed into the gap through the port and form inner layer structure.

Afterwards, PDMS were poured on master 1 and obtain the mold B. Repeat the procedure to punch a hole. The difference is mold B would directly primed PEG hydrogel into the port and do not use master 2. Therefore, there is no channel forming between glass slide and PEG. It is clearly demonstrated in figure 2.

**Fabrication of PEG hydrogel**

As figure 2 step 5 and 8 shown, after finishing mold A and B, they are supposed to expose under UV light 2.5 minutes in order to photocrosslink and form microfluidic networks.When mold A and B finished exposing, two mold were spliced together. Align and contact mold A with mold B, the crosssection is 3D microchannel.

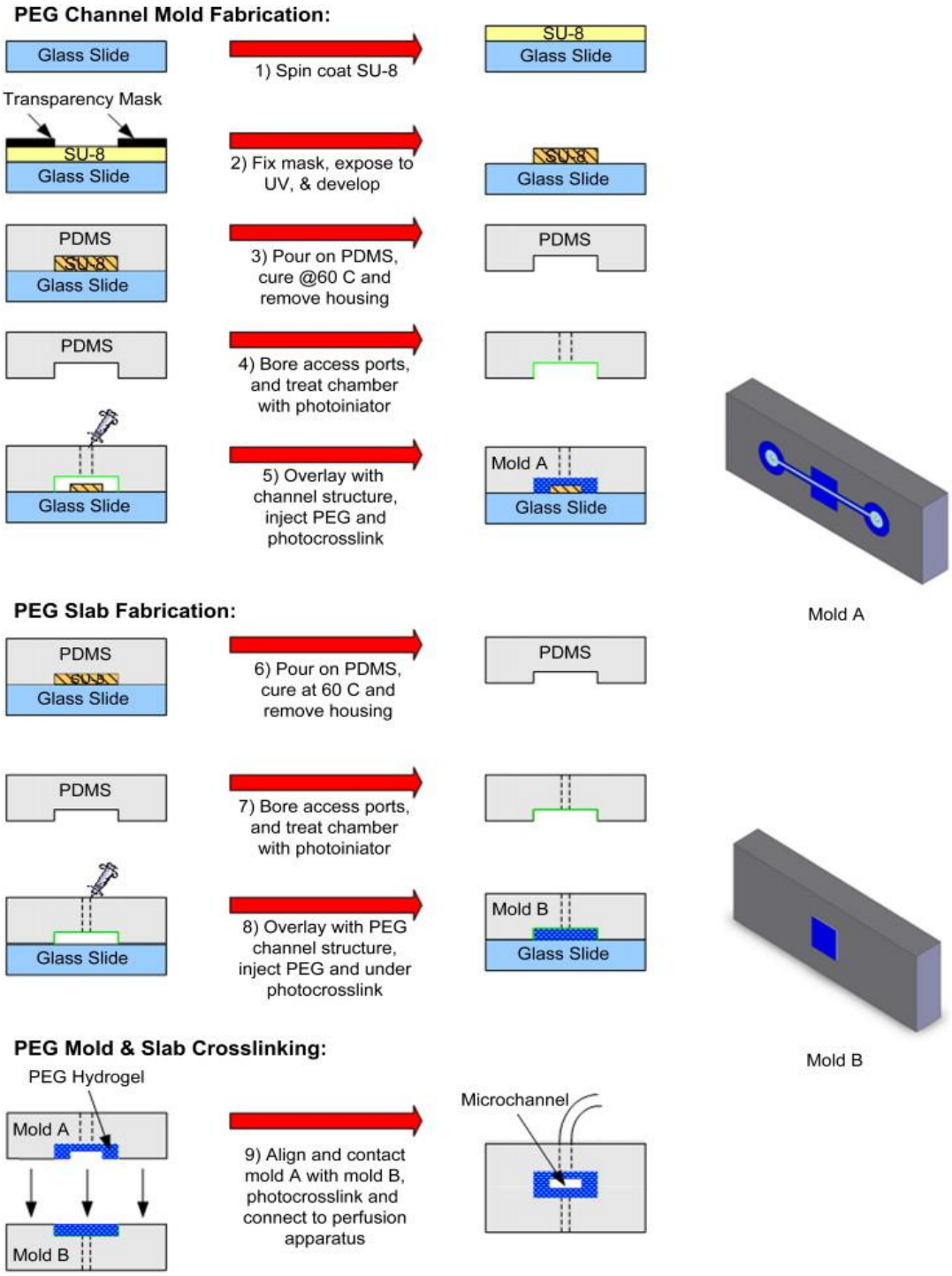


Figure 2. Fabrication procedure of PDMS mold

For stem cell study, another article shown that some cell adhesion peptides such as arignine-glycine-aspartate (RGD) peptides can respond to external stimuli and transmit signals to the cytoskeleton, affecting cell behaviors[27]. It can not only enhance the adhesion of SCs, but also regulate the intracellular mechanisms of

proliferation and differentiation[28]. Considering this, an inspiration proposed whether it is possible to add substances such as RGD peptide to PEGDA to promote cell adhesion, or to study the effect of RGD peptide on SCs through gradient changes. Liu et al. produced a PEG hydrogel with gradient RGD peptide for quantitative analysis[26]. Therefore, when one considers the fabrication of PEG hydrogel, some peptide can be added in this procedure.

## 3.2 Sample Preparation

For animal model, SCs are easy to extract. Rats tend to be sacrificed for obtaining SCs. However, when it comes to human cell extraction, injecting anesthetics and operation may be unacceptable. Although iPSCs method can induce SCs from adult cells, it is still considered as a backup option due to the high cell death rate.

According some authors reported that some tissues such as adipose tissue are alternative sources of mesenchymal stem cells[29],which is also called Adipose-Derived mesenchymal Stem Cells (ADMSCs).Since the human body is rich in adipose tissue, ADMSCs is one of the popular method to obtain mesenchymal stem cells. References illustrated that these SCs can grow and proliferation stably in vitro[30]. Therefore, MSCs study are popular in experiment.

For these SCs extracted *in vivo*, the bone marrow solution was filtered and cenreiduged rather than directly put into the microfluidic device. These cell sample were then cultured in a culture flask at 37℃ in a humidified incubator. Meanwhile, after multiple days of culture, the non-adherent cells ought to be removed. The rest

cells were trypsinized, centrifuged and used for cultivation on PEG hydrogels.

# 4. Biomedical Application

## 4.1 Stem Cell Analysis

Microfluidic device can be used for SC analysis in biophysical regulation, cell-cell interaction,biochemical regulation and adhesive ligands regulation,etc.

The early microfluidic platform as Khademhosseini et al. shown in figure1 picture 1[17]. It originally used microcontact printing method used PDMS and PEG fabricating porous microchannels device to cultivate ESCs and study the interaction and biophysical regulation such as size, shape, and homogeneity.

Picture 2 in figure 1 shown a microdevice consisting of two silicon combs which can precisely locate cell position so that it can study the cell-cell interaction[18]. Contrary to cell-cell interaction, it is difficult to study the effect of adhesion ligands in ECM on the phenotype of SCs. A polymer array synthesis method was proposed for rapid, nanoliter-scale synthesis of biomaterials and the characterization of their interactions with cells[31]. In this experiment, ESCs interacted with substances have been studied. Authors harvested deeply understanding of ESCs behavior including cell-type specific proliferation and differentiation. Another study of ESCs, controlling molecular factors secreted by cells into the surrounding medium, authors studied the influence of paracrine and autocrine signals[32].

## 4.2 Stem Cell Culture

Part of the purpose of cell analysis is cell culture. Unlike cell analysis, SC culture needs to last longer in a microfluidic environment. SCs are highly regulated by the surrounding microenvironment and are highly sensitive to environmental characteristics.

The microfluidic polymer array device mentioned in the previous section can be used for SC culture and the culture rate in the device is higher than that of the static device[31]. In addition, Matsumura et al. developed another snake-shaped microchannel device[32]. The advantage of this device is that it can precisely control the flow of microfluid in the channel, thereby controlling the shear stress imposed on the SCs.Park et al. cultivated abundant neural progenitor cells derived from human ESCs with a continuous cytokine gradient in a microfluidic chamber for 8 days[33].

For a successful attempt based on the SC analysis at biochemical regulation, authors used a porous microfluidic device as mentioned before to simultaneously culture isolated mouse ESCs and mouse embryonic fibroblasts[35]. In this experiment, because authors provided the necessary microenvironmental factors, ESCs remained in an excellent undifferentiated state, which proved the effectiveness of the microenvironmental factors. Even due to the separation effect of the porous membrane, the purity of ESCs is increased.

Meanwhile,a study on the biophysical regulation of SCs is low shear stress. After storing MSCs at low temperature and then returning to room temperature, MSCs still have the potential of differentiation and adhesion under the treatment of low shear

stress[36].

## 4.3 Stem Cell Differentiate

Stem cells can produce various kinds of cell types. As the introduction mentioned, SCs have been used in clinic for some regeneration. When authors want to differentiate cells, the first thing should be considered is what kind of cells are desired to produce.

Traditional culture methods tend to use inducing factors to differentiate SCs in a 2D environment because it cannot provide a 3D microenvironment. It has been confirmed that an appropriate microenvironment can not only promote the maintenance of SCs, but also regulate the differentiation of SCs to achieve homeostasis. Therefore, in addition to adding soluble factors, the microfluidic device can precisely control the microenvironment of SCs, including flow conditions,ECM, biomechanics,etc. to guide the differentiation of stem cells.

Some authors have developed a hybrid microfluidic system to generate a dynamic microenvironment[37]. The conclusion is that compared with the conventional system, the specific differentiation of SCs in a dynamic microenvironment is significantly higher.

As mentioned in section 4.1, paracrine signals may affect SC activity. The microfluidic array platform was used to co-culture NSCs and MSCs, where MSCs were genetically engineered to overexpress glial cell-derived neurotrophic factor[38]. When co-cultured, the glial differentiation of NSCs decreased, and at the same time,

the differentiation of neuronal cells increased.

Besides the signal stimulation, there are other factors affecting SCs differentiation. Extremely low shear stress enhanced MSCs differentiate osteogenic cell which is belong to biophysical stimulation[39]. Multiple stimuli can also applied to stem cell simultaneously, for example, combing chemical stimulation and pure pressure stimulation has been proved that is available to promote SC differentiation. By stimulating with 1-methyl-3-isobutylxanthine and different shear forces promoted the differentiation of PSCs into nerve cells[40].

### 4.3 Extracellular Scaffolds

In recent years, some new functional hydrogels have been developed to construct extracellular scaffolds for SC culture. Microfluidic devices can use hydrogels as a more physiologically 3D matrix. By utilizing microchips for precise manipulation, cell-containing hydrogels can be fabricated to diverse geometric shapes and structures.

SCs incorporated during the formation of scaffold, a 3D notwork can be built which promotes cell migration and proliferation and leads to rapid regeneration of skin tissue in the experiment[41].By constructing ECM components oriented along the flow direction, neurons differentiated from NSCs grow directionally[42].

### 4.4 Tissue Engineering

Organs-on-a-chip are based on microfluidic cell culture to simulate the physiological functions of tissues and organs just using the smallest functional unit

that can summarize the function of tissue and organ.Precisely controlling the differentiation of SCs in a microfluidic microenvironment can realize tissue engineering and the development of organs-on-a-chip[43].

In order to realize heart-on-a-chip, the patient's cardiomyocytes were extracted, using IPSCs technology combined with tissue engineering to produce a similar condition in the body on the chip so that the pathogenesis were identified successfully[44].

Another example of organ-on-a-chip is fabricated by artificial bone and living bone marrow. The bone marrow-on-chip had an amazing hematopoietic function *in vitro*[45].

Authors constructed a 3D functional and perfusible microvascular network which is composed of human endothelial cells and MSCs phenotype transitioning to parietal cells. Unfortunately, this microvascular network did not have a functional role. After adding angiopoietin, the system generated perfusible capillaries[46].

## 5. Future Discussion

Retrospecting articles, microfluidic techniques still have great potential especially on the extracellular scaffolds and tissue engineering.It is expected that more and more microfluidic devices and microchannel design. The future development may focus on lab-on-a-chip which is towards monolithic organs and clinical applications.

Although some microvascular network and human skin can be obtained according some research mentioned above, the main problem is that due to the size

limitations, it is difficult to realize large tissues and organs in microfluidic channels, which may require to combine with other techniques. Although advanced microfluidic technology faces some challenges, due to its 3D properties, it will become a useful tools for SC study.

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