

***In vitro* investigation of gas embolism in microfluidic networks mimicking microvasculature**

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Supplementary Information

1. Dimensions of the mimicked microvasculature

The dimensions of the mimicked microvasculature, namely linear T-junction channel, linear Y-junction channel, and bifurcating honeycomb microchannels, were illustrated in Fig. S1. The linear channels with two types of junctions with three different widths, namely 20 μm , 40 μm and 60 μm . The width of the honeycomb bifurcation network was 30 μm .

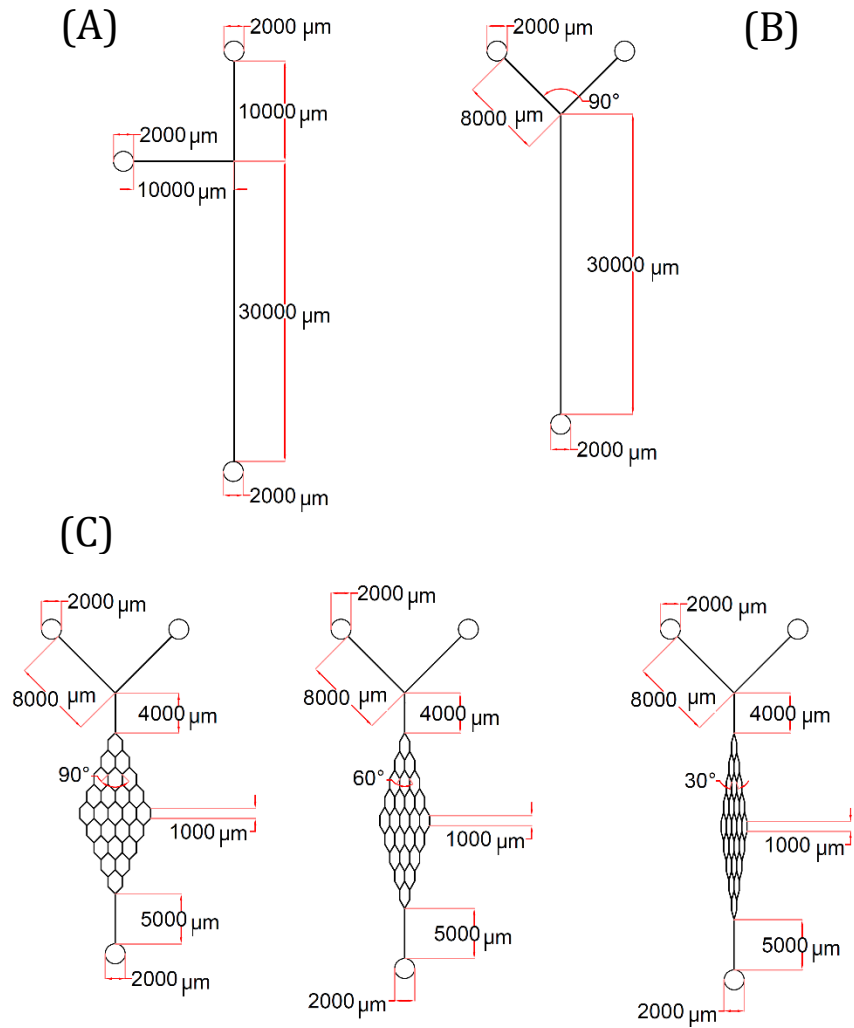


Fig. S1. The details of mimicked microvasculature dimensions. (A) T-junction single microchannel; (B) Y-junction single microchannel; (C) honeycomb-like bifurcation microvascular channel.

2. Master fabrication

The microvasculature designs were created with Fusion 360 and printed on a mylar sheet to fabricate a photomask (made by CAD/Art Services, Inc.). A SU-8 negative photoresist 2050 (Kayaku Advanced Materials, Inc.) layer with the thickness of 40 μm was coated on 4-inch silicon wafers (Ultra-flat single-side polished silicon substrates, 1000 μm thickness, Alpha Nanotech Inc.) by a spin coater (WS-650-23 B spin coater, Laurell Technologies Corporation). The silicon wafer, photomask alignment and UV exposure were performed using a mask aligner system (OAI, Hydralign Series 2000). The photomask was placed onto the photoresist spin-coated silicon wafer and UV-exposed for 55 seconds. The unexposed part of the photoresist layer was then removed by dissolving in the SU-8 developer (Kayaku Advanced Materials, Inc.). Finally, the silanization treatment improved bonds across the interface between mineral and organic components ¹. The schematic of the masters' fabrication procedure and PDMS replication of microvasculature are shown in Fig. S2.

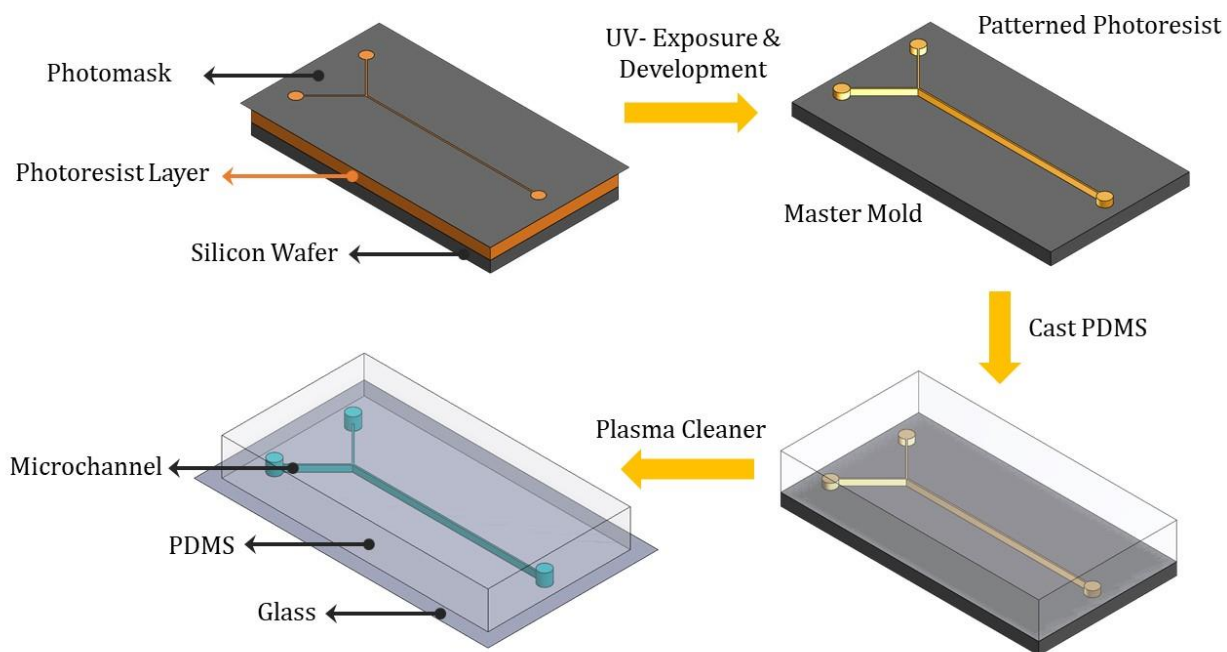


Fig. S2. The schematic of the masters' fabrication process of the microvasculature.

3. Polydimethylsiloxane (PDMS) replication moulding

Polydimethylsiloxane (PDMS, Sylgard Silicone elastomer 184, Dow Corning Corp. Midland, MI.) and curing agent were mixed in a 10:1 ratio (weight by weight). PDMS mixture was poured onto the fabricated master and then degassed to remove the air bubbles. The curing process was carried-out overnight at 60 $^{\circ}\text{C}$ in an oven. After

curing, the PDMS with microchannels networks was removed from the silicon wafer, and the holes for inlets and outlets were punched (1.5mm diameter punctures were used). The PDMS was treated by plasma cleaner (PDC-32G, Harrick plasma) to form an irreversible bond, and then a piece of glass was used to seal the microfluidic channels. The locked microfluidic devices were then used for the experiments.

4. Flow patterns of fluids in T-junction and Y-junctions:

The representative panel of the air bubble, liquid slug patterns observed with different flow ratios and equivalent concentrations of the hematocrit for both T-junction Y-junction.

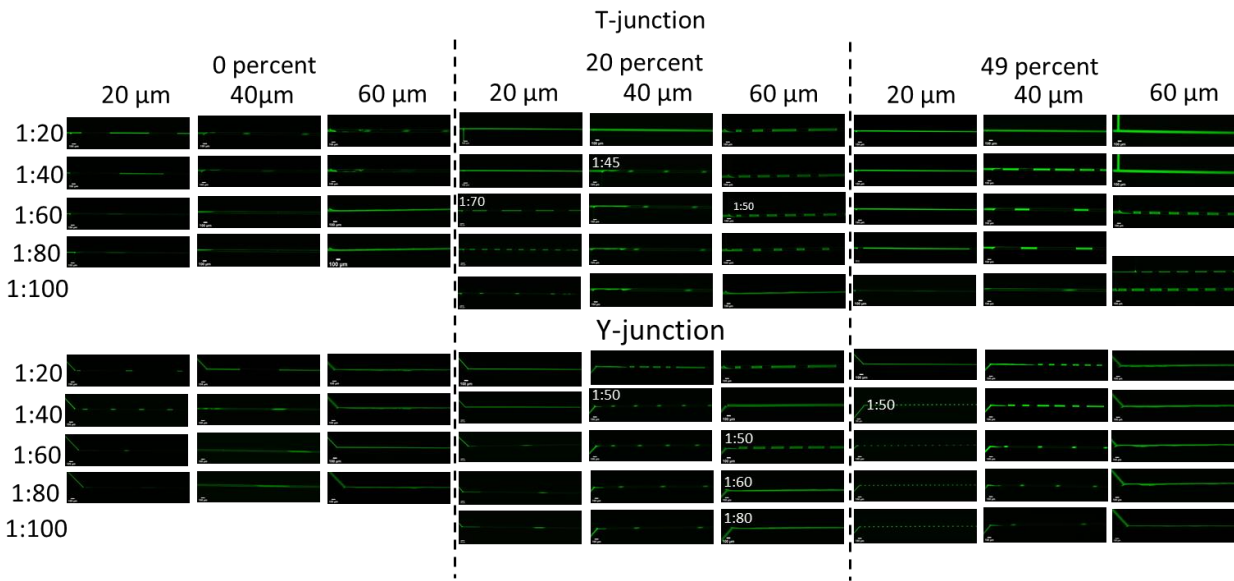


Fig. S3. The flow patterns of air bubbles and liquid slugs in T-junction and Y-junction microchannels (20 μm , 40 μm and 60 μm) at the different liquid to airflow rates ratios and equivalent concentration of the hematocrit (0%, 20% and 46%).

5. MATLAB codes for image analysis

The estimation of the flow velocity of the liquid slug and air bubbles were estimated using the MAT lab codes below. All image analyses were performed using the ImageJ as detailed in the materials and methods, while the velocity calculations were performed using the codes below.

```
clear
clc
location = '';
v = VideoReader(location);
numberF=v.NumFrames;
% I1 = imcrop(videoFrame , [X_barycentre-h/2 Y_barycentre-h/2 h h]);
% v.CurrentTime=time;
rateF=v.FrameRate;
```

```

frametime=1/rateF;
timetotal=v.Duration;
widthvideo=v.Width;
heightvideo=v.Height;
Detection=[];
Detection1=[];
noDetection=[];
kalmanFilter = [];
DetectionTotal=[];
for nf=1:numberF
    frame = readFrame(v);
    frame = imadjust(frame,[0 0.50]);
    grayImage = rgb2gray(im2single(frame));
    grayImage=imgaussfilt(grayImage);
    [a b]=size(grayImage);
    BW=imbinarize(grayImage);
    se = strel('disk',3);
    BW= imopen(BW,se);
    se = strel('square',6);
    bw=imclose(BW,se);
    p=find(bw(:,1));
    [a1 b1]=size(p);
    for i=1:b1
        B=find(bw(:,i));
        [dt bt]=size(B);
        if dt==0;
            bw(:,i)=1;
        elseif dt<=10;
            bw(:,i)=1;
        else
            bw(:,i)=0;
        end
    end
    if p~0;
    for i=1:p(1)-1
        bw(i,:)=0;
    end
    for i=p(a1)+1:a1
        bw(i,:)=0;
    end
end
se = strel('square',2);
bw=imclose(bw,se);
%% Detection
[L,n] = bwlabel(bw);
stats =
regionprops(L,'area','Centroid','MajorAxisLength','MinorAxisLength','Bound
ingBox');
allAreas =cat(1,stats.Area);
allBoundingBox=cat(1,stats.BoundingBox);
if n~0;
MainPoint=allBoundingBox(:,1)+allBoundingBox(:,3);
FrameN=[nf];
if n>=1;

```

```

for bd=1:n
    if MainPoint(bd)>=2;
        Detection1=cat(1,FrameN,FrameN,MainPoint(bd),allBoundingBox(bd,3));
        isObjectDetected = true;
        Detection=cat(2,Detection,Detection1);
    else
        isObjectDetected = false;
    end
end
else
    isObjectDetected = false;
end
if isObjectDetected == true;
    DetectionTotal=cat(2,DetectionTotal,Detection);
    Detection=[];
else
    noDetection=cat(1,noDetection,FrameN);
end
end
end
xlswrite('40um_Tjunc_20per_ratio80-1.xlsx',DetectionTotal);

```

Reference

1. K. Subramani, in *Emerging nanotechnologies for manufacturing*, Elsevier, 2015, pp. 279-293.