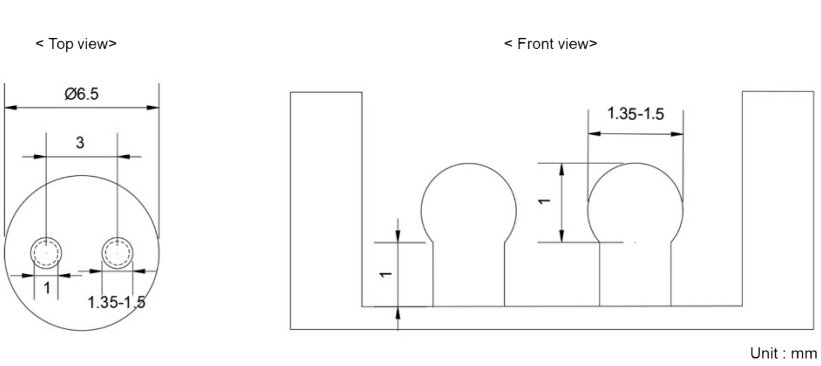
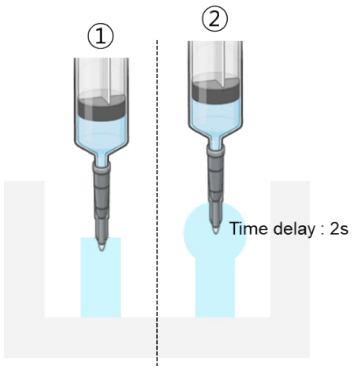
Supplementary Data

Table of Contents

(a)



(b)



(c)

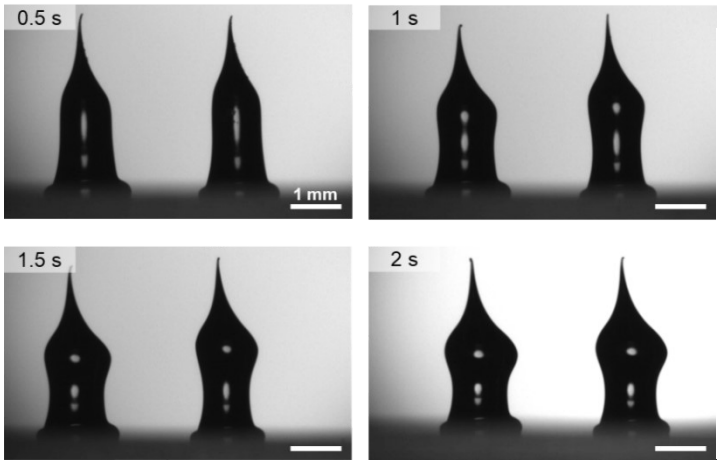
Supplementary Figure 1. Quantitative specifications of the 96-well plate and mushroom-type pin anchors

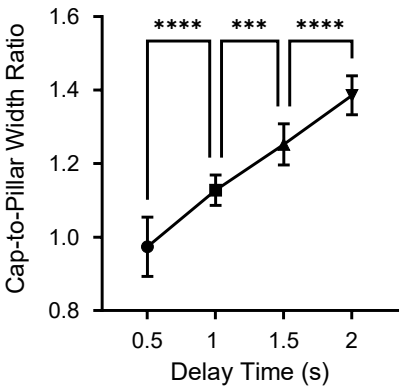
(a) Schematic of the well dimensions of the 96-well plate

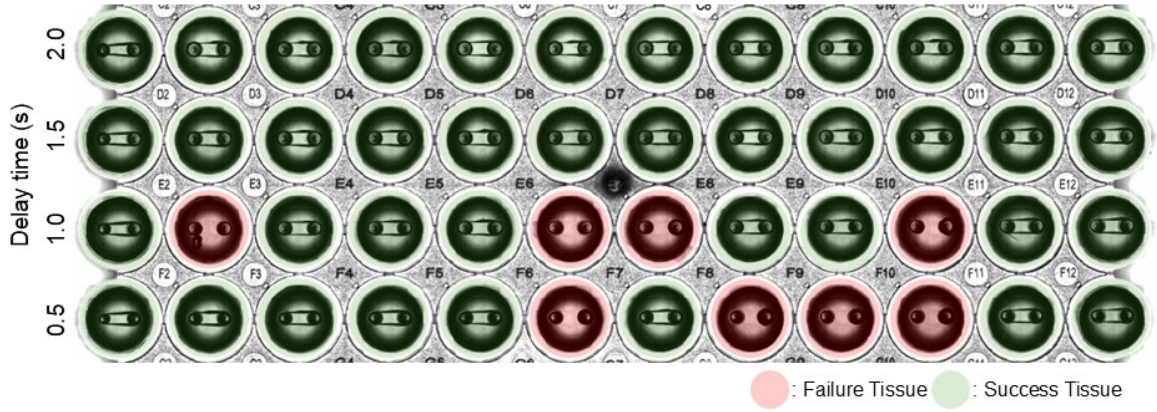
(b) Schematic illustration showing the detailed dimensions of the mushroom-type pin anchor, designed and drawn using CAD software.

(c) Schematic illustration of the anchor fabrication process controlled by the printing time delay. The cap size of the anchor is not defined by a fixed dimension but is determined by the printing time delay; a 2 s delay was used during fabrication, resulting in a cap length of approximately 1.35-1.5 mm depending on various situational factors.

(a) (b)







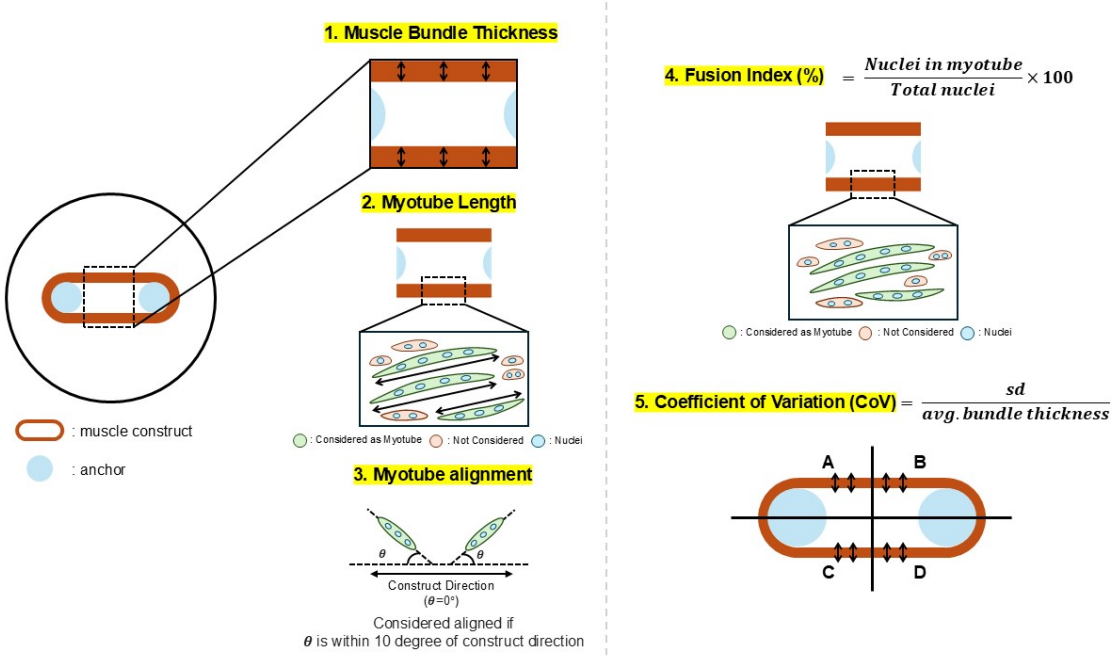
(c)

Supplementary Figure 2. Optimization of mushroom-type anchor fabrication by controlling the printing time delay

(a) Optical tensiometer images of mushroom-type pin anchors fabricated with different printing time delays.

(b) For each anchor image, the maximum width was defined as the cap width and the minimum width as the pillar width, and the ratio between these two values was calculated (n = 10). Data are expressed as mean ± SD (***$P < 0.001$, ****$P < 0.0001$).

(c) Representative images of 3D skeletal muscle (SKM) tissues fabricated using anchors with different printing time delays (n = 12, day 3).



Supplementary Figure 3. Image analysis method

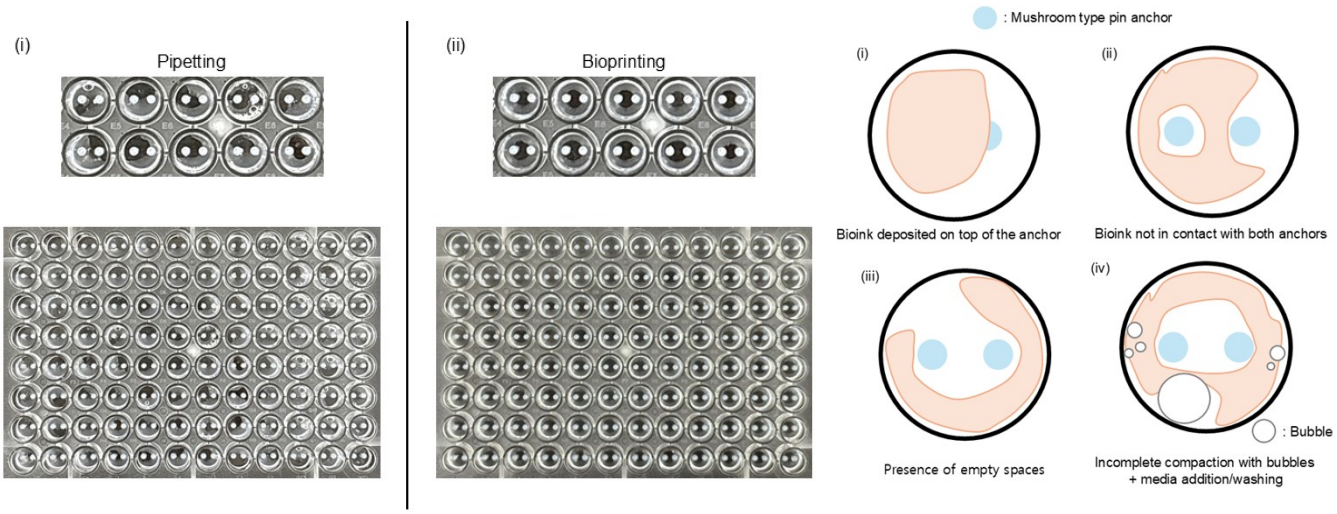
1. The thickness of each bundle was measured at all defined positions.

2. The length of myotubes meeting the criteria defined in this study was measured.

3. Only myotubes oriented within ±10° relative to the 0° reference axis were included in the analysis.

4. The percentage of nuclei within myotubes was calculated relative to the total number of nuclei in the selected image.

5. Muscle tissues were divided into four regions, and muscle thickness in each region was measured to calculate the mean value and standard deviation.



(a) (b)

Supplementary Figure 4. Images immediately after bioink embedding using each method (20-25 µL) and causes of failure tissue formation during pipetting

(a) Representative images of the plate immediately after bioink embedding using pipetting and bioprinting methods

(i) Image of the entire 96-well plate immediately after bioink embedding using pipetting.

(ii) Image of the entire 96-well plate immediately after bioink embedding using bioprinting.

(b) Schematic illustration showing the causes of failure tissue during the fabrication of the skeletal muscle plate using pipetting (floating-off and slipping-off).

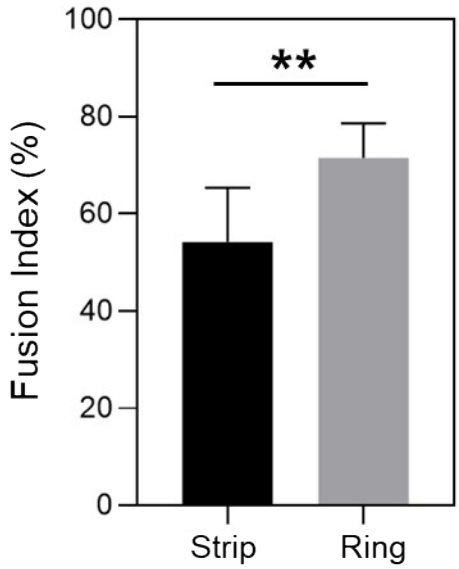
(i) When the bioink is deposited onto one or both anchors.

(ii) When the bioink fails to fully surround both anchors and instead wraps around only a single anchor.

(iii) When the bioink surrounds both anchors but fails to make contact between ink regions, resulting in the formation of empty spaces.

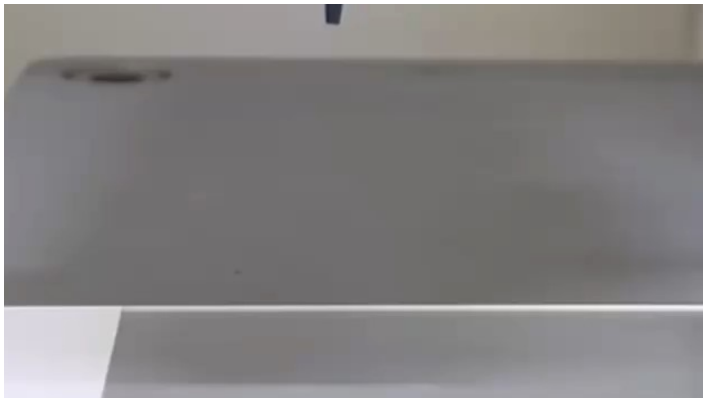
(iv) When bubbles are generated due to pipetting errors, leading to complete detachment from the well bottom during media addition or PBS washing after gelation, or when bubble collapse results in the formation of empty spaces similar to case (iii).

(a)



Supplementary Figure 5. Comparison of fusion index between strip-shaped and ring-shaped tissues

(a) Graph showing the fusion index of strip-shaped and ring-shaped tissues (n = 3). Data is expressed as mean ± SD (**$P < 0.01$).



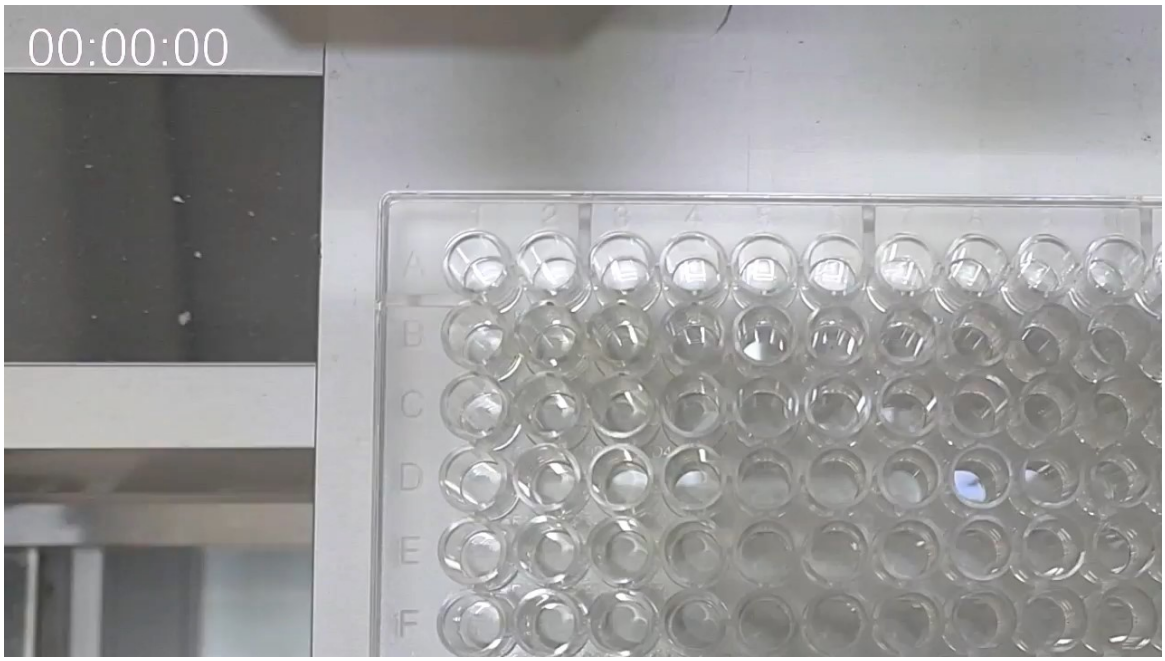
(a)

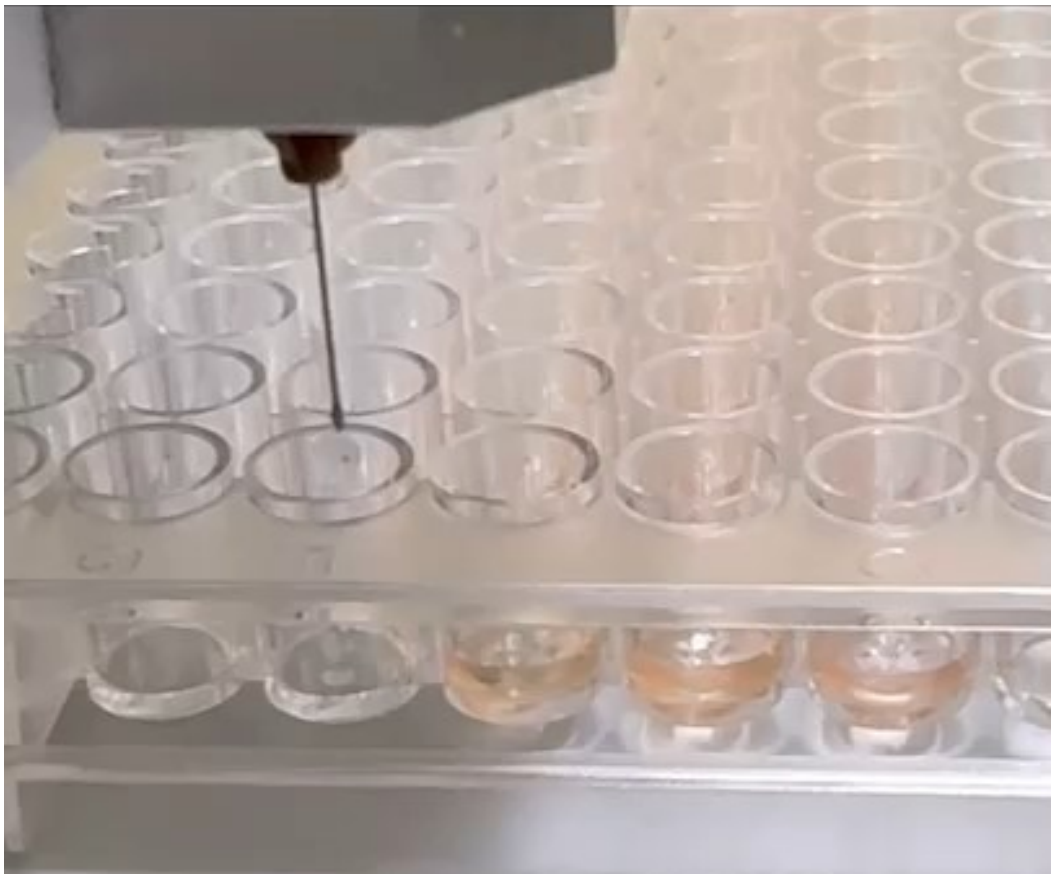
(b)

Supplementary Video 1.

(a) Anchor printing process (4× speed).

(b) Anchor printing across the entire 96-well plate (32× speed).





Supplementary Video 2.

Printing of muscle cell–laden bioink using a 3D bioprinter (4× speed).

Supplementary source code

;G90 indicates absolute coordinates

;G91 indicates relative coordinates

;G1 indicates movement of printing head

;G4 indicates a dwell (pause) for a specified duratio

;M10 activates material dispensing (pneumatic valve on)

;M11 deactivates material dispensing (pneumatic valve off)

;G3 indicates counterclockwise circular interpolation (arc movement) of the printing head

;X and Y indicate printing head movement along each horizontal axis

;Z, a, b indicate each printing head movement along vertical axis

;F indicates movement speed of printing head. Unit is mm/min

;I specifies the X-axis offset from the start point to the center of the arc

;J specifies the Y-axis offset from the start point to the center of the arc

;Mushroom-type pin anchor printing

;NOZZLE 22TPND , 140-170KPA SE1700 (silicone)

;The following code prints a single line (12 wells).

;To fabricate the entire AnchorPlate96, this sequence should be repeated eight times

;Each line is spaced 9 mm apart

; CODE STARTS

G90

;Please find the start position and insert it below

G1 X0 Y0 1000

G1 Z0F1000

G91

M10

G4 X1.5

```
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F1000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
```

```
G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
```

```
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
```

```
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
```

```
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
```

```
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000
```

```
G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

G1 X-6 F3000
G1 Z-11.5 F3000

G91
M10
G4 X1.5
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000
G1 X-3 F3000
G1 Z-11.5 F3000
M10
G4 X1.5
```

```
G1 Z1.5 F22
G4 X2
M11
G1 Z10 F1000

M10
```

Supplementary source code

;C2C12-laden bioink printing

;NOZZLE 27G, 10-15KPA 0.5wt% COLLAGEN

;The following code prints a single line (12 wells).
;To fabricate the entire AnchorPlate96, this sequence should be repeated eight times
;Each line is spaced 9 mm apart

; CODE STARTS
G90

;Please find the start position and insert it below
G1 X0 Y0 1000
G1 Z0F1000

G91
M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200

```
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
```

```
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
```

```
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
```

```
G1 X-9 F2500
G1 Z-15 F2500

M10
G4 X0.3
G3 X5.7 Y0 I2.85 J0 F200
G3 X-5.7 Y0 I-2.85 J0 F200
G4 X0.1
M11
G1 Z15 F2500
;
```