Electronic Supplementary Material (ESI) for Lab on a Chip.

Silicon-nanowire based attachment of silicon chips for mouse embryo labelling

Sara Durán[a], Sergi Novo [b], Marta Duch[a], Rodrigo Gómez-Martínez[a], Marta Fernández-Regúlez[a], Álvaro San Paulo[a], Carme Nogués[b], Jaume Esteve[a], Elena Ibañez [b], José Antonio Plaza[a]

[a] Instituto de Microelectrónica de Barcelona, IMB-CNM (CSIC), Campus UAB, 08193, Cerdanyola, Barcelona, Spain.

[b] Department of Cellular Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain

Electronic Supplementary Information

Supplementary methods

M1. Nanowire growth method assisted by catalyst gold deposition.

M2. Mouse embryo collection.

M3. Embryo sample processing.

Supplementary figures

S1. The design of the barcodes.

S2. The calculated applied force between the embryo and the barcodes during the Rolling method.

S3. The calculated applied force between the embryo and the barcodes during the Pushpin method.

S4. Analyzed wires during the studies of strength and radius of curvature reached by silicon nanowires.

Supplementary videos

V1. Rolling method.

V2. Pushpin method.

Supplementary methods

M1. Nanowire growth method assisted by catalyst gold deposition

For galvanic displacement deposition of the gold catalyst nanoparticles, the devices were immersed in a microemulsion formed by an aqueous solution which contains potassium gold (III) chloride ($KAuCl_4$) and a low concentration of hydrofluoric acid (HF) and an organic solution made of n-heptane and a surfactant, dioctyl sodium sulfosuccinate (AOT). The mixture of this two solutions results in a microemulsion of inverse micelles. The diameter of the resulting gold nanoparticles is directly proportional to the diameter of the micelles which is proportional to the ratio between water and surfactant molecules, providing a way of controlling the diameter of the nanowires. The silicon nanowires were grown in a chemical vapor deposition tube furnace at 750 - 850ºC by a mixture of $SiCl_4$ precursor gas (30 sccm - 60 sccm), BBr_3 dopant gas (0 sccm - 1 sccm) and Ar - 10% H2 carrier gas (250 sccm - 300 sccm) for 60 - 90 seconds.

M2. Mouse embryo collection

Animal care and procedures required in this study were carried out according to the protocols approved by the Ethics Committee on Animal and Human Research of the Universitat Autònoma de Barcelona and by the Departament d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Pronuclear embryos were obtained from superovulated hybrid B6CBAF1 (C57BL/6JxCBA/J) female mice (age, 8-12 weeks) injected with 5 IU of pregnant mare serum gonadotropin (Intervet, Spain) followed, 48 h later, by 5 IU of human chorionic gonadotropin (Farma-Lepori, Spain) and mated with males of the same strain. Embryos were collected from the oviducts 25 h after human chorionic gonadotropin administration, and incubated during 5-10 min at 37°C in Hepes-buffered potassium simplex optimized medium (H-KSOM),[1] 150 U/ml of

hyaluronidase (Sigma, Spain) for the dispersion of cumulus cells. After denudation, embryos were washed twice in fresh H-KSOM.

M3. Embryo sample processing for FESEM

Embryos with barcodes attached to their ZP were washed three times in 0.1 M cacodylate buffer and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for at least 2 h at room temperature (RT). Next, fixed embryos were washed three times for 5 minutes in 0.1 M cacodylate buffer and post-fixed during 2 h at RT by osmification using 1% osmium tetroxide in 0.1 M cacodylate buffer. After two rinses for 10 min in distilled water, embryos were dehydrated in a series of increasing ethanol concentrations until 100%. Finally, they were critical point-dried using CO_2 (CPD 030 critical point dryer Bal-Tec) and mounted onto aluminum stubs. The samples were observed under a field emission scanning electron microscope (FSEM; Merlin Zeiss, Germany).

Supplementary figures

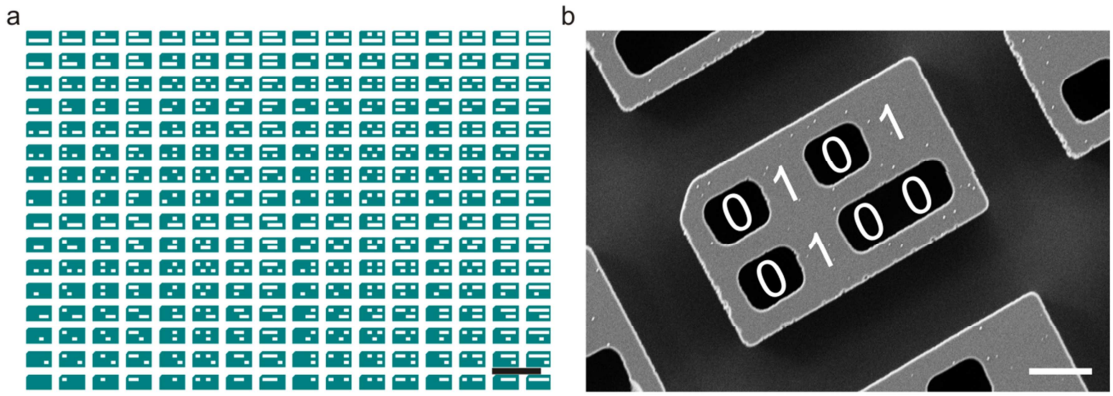
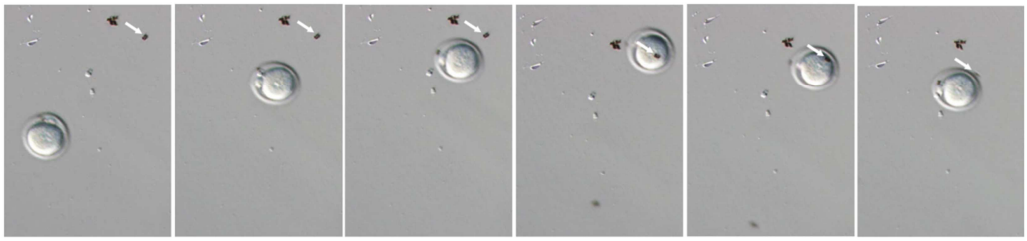


Figure S1. The design of the barcodes. a) Schematic view of the 256 different barcodes and b) a SEM image showing a fabricated polysilicon barcode at wafer level. They represent data by rectangular bits (full rectangle bit = 1, empty rectangle bit = 0). Black scale bar = 20 µm, White scale bar = 2 µm.

a



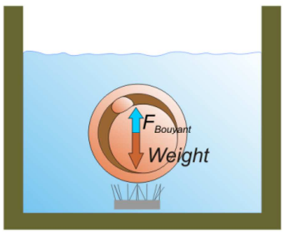
b

Embryo (n)	Time (s)	Length (m)	Terminal Velocity (m/s)	Radius of the Embryo (m)	Density of the Embryo (Kg/m^3)	Volume of the Embryo (m^3)	Mass of the Embryo (Kg)
1	19.32	0.01	$5.18 \cdot 10^{-4}$	$5.19 \cdot 10^{-5}$	1096	$5.87 \cdot 10^{-13}$	$6.43 \cdot 10^{-10}$
2	18.10	0.01	$5.52 \cdot 10^{-4}$	$5.01 \cdot 10^{-5}$	1109	$5.25 \cdot 10^{-13}$	$5.83 \cdot 10^{-10}$
3	20.04	0.01	$4.99 \cdot 10^{-4}$	$5.18 \cdot 10^{-5}$	1093	$5.82 \cdot 10^{-13}$	$6.37 \cdot 10^{-10}$
4	24.18	0.01	$4.14 \cdot 10^{-4}$	$5.28 \cdot 10^{-5}$	1076	$6.16 \cdot 10^{-13}$	$6.63 \cdot 10^{-10}$
5	20.79	0.01	$4.81 \cdot 10^{-4}$	$5.16 \cdot 10^{-5}$	1091	$5.76 \cdot 10^{-13}$	$6.28 \cdot 10^{-10}$
6	25.94	0.01	$3.86 \cdot 10^{-4}$	$5.03 \cdot 10^{-5}$	1078	$5.33 \cdot 10^{-13}$	$5.74 \cdot 10^{-10}$
7	18.37	0.01	5.4410^{-4}	$5.03 \cdot 10^{-5}$	1107	$5.34 \cdot 10^{-13}$	$5.91 \cdot 10^{-10}$
8	19.00	0.01	$5.26 \cdot 10^{-4}$	$5.06 \cdot 10^{-5}$	1102	$5.44 \cdot 10^{-13}$	$5.99 \cdot 10^{-10}$
Average	20.72	0.01	$4.90 \cdot 10^{-4}$	$5.12 \cdot 10^{-5}$	1094	$5.62 \cdot 10^{-13}$	$6.15 \cdot 10^{-10}$
Errror (±)	2.85*	0.001	$0.83 \cdot 10^{-4}$	$0.99 \cdot 10^{-5}$ *	15	$0.33 \cdot 10^{-13}$	$0.37 \cdot 10^{-10}$

* *Standard deviation*

$$\rho_{Emb} = \frac{9 \cdot vel \cdot \mu_{PBS}}{2 \cdot r_{Emb}^{\ 2} \cdot g} + \rho_{PBS} \longrightarrow \begin{array}{l} Weight_{Emb} = \rho_{Emb} \cdot Volume_{Emb} \cdot g \\ F_{Bouyant} = Volume_{Emb} \cdot \rho_{KSOM-H} \cdot g \end{array} \Bigg| \; F_{Rolling} = Weight_{Emb} - F_{Bouyant}$$

c



d

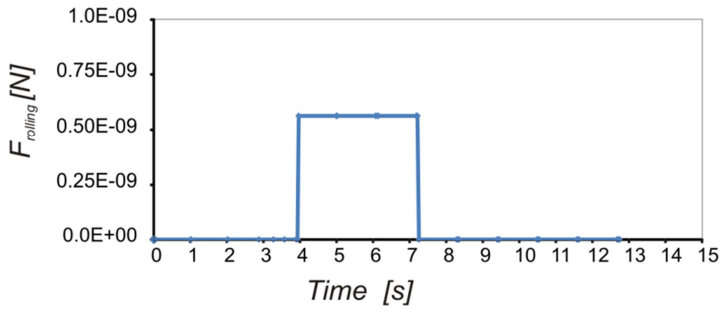


Figure S2. The calculated assumed force between the embryo and the barcodes during the Rolling method. a) Extracted frames from the supporting Video1 taken with an inverted optical microscope showing an embryo overpassing a barcode (white arrows) with the nanowires facing upwards. b) The weight of the embryos were calculated by determining their dimensions on an optical microscope (Volume = $4/3 \cdot \pi \cdot r_{Emb}^{\ 3}$, where r_{Emb} is the radius of the embryo) and calculating

their average density, ρ_{Emb}. The ρ_{Emb} of the mouse embryo was calculated by using a method equivalent to the falling sphere viscometers. Eight embryos (spherical particles) were placed individually in PBS medium and allowed to descend through the liquid until they reached terminal velocity, *vel*. The terminal velocity was calculated by the time they took to descend 10 mm. The average density of the embryos, ρ_{Emb}, was given by the Stoke´s law, where μ_{PBS} was the viscosity of the PBS (0.001002 Kg/m·s); ρ_{PBS} was the density of the PBS (1007.7571 Kg/m3); and g was the gravitational acceleration. During the Rolling test the embryos were immerged in KSOM-H medium, with an experimentally determined density of 992 ± 14 kg/m^3, which exerted an upward force, buoyant force. The force between the embryos and the barcodes was calculated by subtracting the buoyancy force, from the weight of the embryo. c) Two forces were applied to the embryo: its weight due to the gravity and the buoyant force. d) A step function was assumed for the applied force between the embryo and the barcode, as the force was only applied while the embryo was over the barcode.

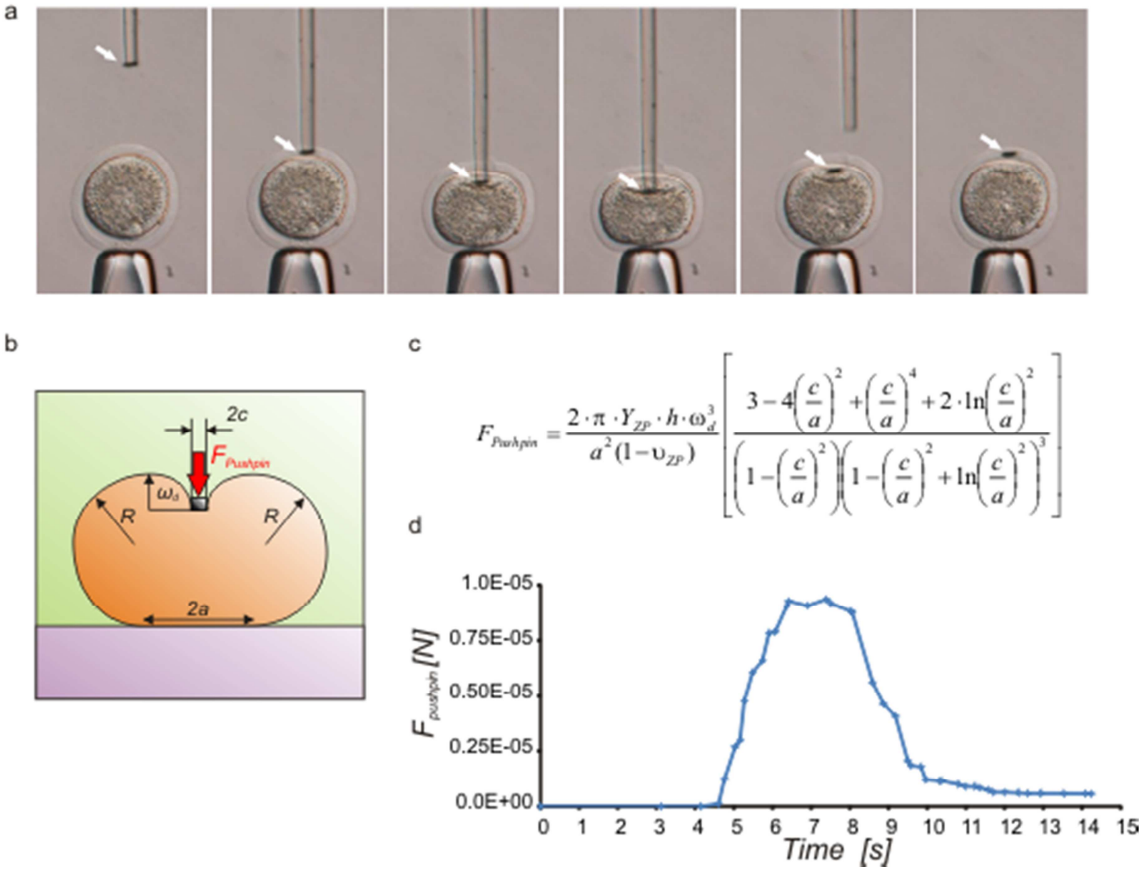


Figure S3. The calculated applied force between the embryo and the barcodes during the Pushpin method. a) Extracted frames from the supplementary Video 2, taken on an optical microscope, showing a barcode (white arrow) with the nanowires towards the embryo and fixed at the tip of a pipette pushing the ZP. The applied force between the barcode and the embryos was approximated by the biomechanical membrane model described by Sun et al. Elastic modulus[2] (Y_{ZP}) and the Poisson ratio,[3] (υ_{ZP}) were set to 42200 Pa and 0.5, respectively. b) The geometric parameters, a and ω_d, were measured from the images captured in the experiments (a). The constant values were h = 6.6 ± 0.1 µm for the thickness of the ZP and c = 5.0 ± 0.1 µm as effective indenter radius. c) Sun et al. Pushpin force formula was applied to calculate[2] d) the corresponding force function between the embryo and the barcode.

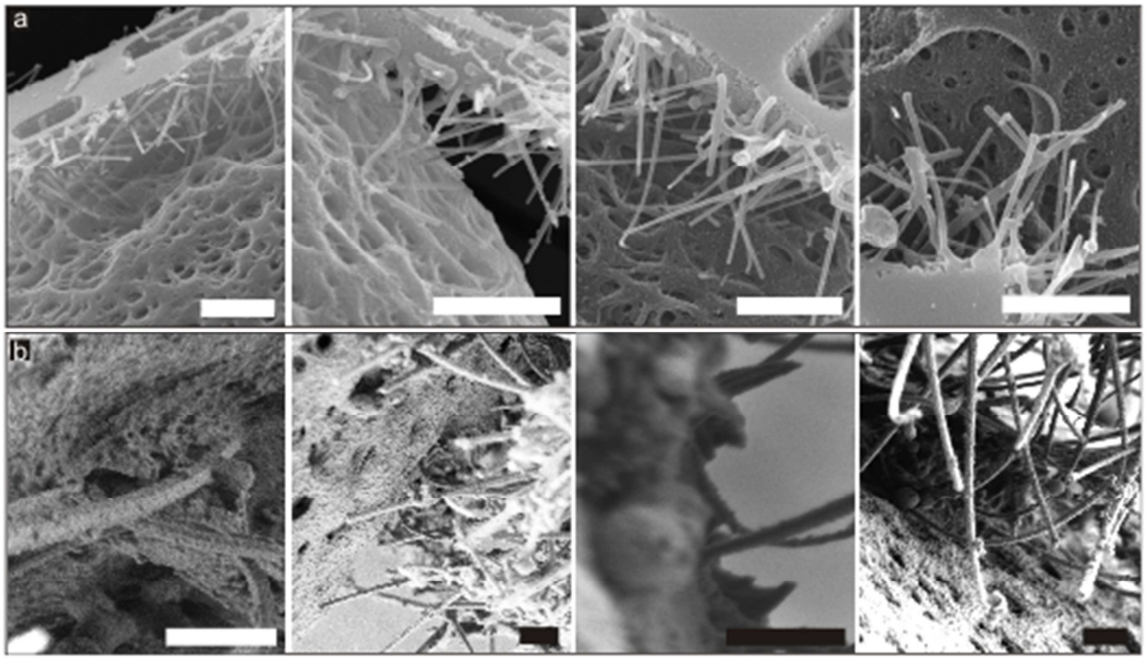


Figure S4. Samples of silicon nanowires during the studies of strength and radius of curvature. FESEM images of attached barcodes to the ZP showing bent nanowires. a) Scale bars = 2 µm. b) Scale bars = 500 nm.

REFERENCES

(1) Biggers, J. D.; McGinnis, L. K.; Raffin, M. Biol Reprod. 2000, 63, 281-293.

(2) Sun, Y.; Wan, K-T.; Roberts, K. P.; Bischof, J. C.; Nelson, B. J. IEEE Trans. Nanobioscience 2003, 2 (4), 279-286.

(3) Vinckiera, A.; Semenza, G. FEBS Letters **1998**, 430, 12-16.