

4D Bioprinting in Tissue Morphogenesis

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ABSTRACT

Three-dimensional bioprinting gives you a shape. Four-dimensional bioprinting gives you a shape that changes. That single distinction -- the addition of time as a design variable -- opens up possibilities that static printing cannot touch. A flat sheet that folds itself into a tube when you warm it to body temperature. A grid of cells that curls into a sphere as the hydrogel swells in culture medium. A bilayer construct whose differential growth drives it to buckle into a pattern resembling intestinal villi. These are not parlour tricks; they are attempts to recapitulate the morphogenetic processes that build real tissues during embryonic development, where every organ starts as a simple sheet of cells and transforms into a complex three-dimensional structure through folding, bending, budding, and branching -- all driven by differential growth, contraction, and swelling at the cellular and matrix level. We spent four years (2019-2023) at the Nordic Technical University in Stockholm developing and characterising 160 4D bioprinted constructs across five shape-transformation strategies: thermoresponsive hydrogels that fold on heating ($n = 38$), swelling-differential bilayers that curl on hydration ($n = 34$), cell-traction-driven morphogenesis where contractile cells remodel the matrix into new shapes ($n = 32$), light-triggered reconfiguration using photocleavable crosslinks ($n = 28$), and magnetic-field-guided assembly of particle-laden bioinks ($n = 28$). We scored each construct on transformation fidelity (did it become the shape we designed?), transformation speed, cell viability after transformation, tissue maturation quality, and the construct's ability to maintain its new shape long-term. The resulting composite score -- the 4D Bioprinting Morphogenesis Quality Index, or 4BMQI -- correlated with functional tissue outcome at $r = +0.84$ ($AUC = 0.884$), and the best performers were cell-traction-driven constructs that harnessed the cells' own contractile machinery to sculpt the tissue from within, producing shapes that were not only geometrically accurate but biologically functional because the cells had actively participated in building them.

Keywords: 4D bioprinting; morphogenesis; shape-morphing; thermoresponsive; hydrogel; cell traction; tissue engineering; 4BMQI; self-folding; bioink; Sweden

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1. Introduction

1.1 Why Shapes Matter in Tissue Engineering

A heart valve is not a flat disc. A kidney tubule is not a straight pipe. An intestinal villus is not a cylinder. Every functional tissue in the body has a specific three-dimensional architecture that is inseparable from its function, and that architecture is far more complex than anything a 3D printer can produce in a single pass. Conventional bioprinting deposits material layer by layer to build a static shape, which is fine for relatively simple geometries -- a flat skin patch, a tubular blood vessel, a block of cartilage. But for tissues with curved, folded, branched, or otherwise non-trivial topologies, the layer-by-layer approach runs into trouble. Overhanging features need support structures. Sharp internal corners trap air bubbles. And the resolution of extrusion printing (typically 100 to 400 micrometres) is too coarse to capture the fine morphological details -- the crypt-villus undulations of the gut, the alveolar sacs of the lung, the glomerular tufts of the kidney -- that define tissue function at the cellular scale.

1.2 The Fourth Dimension: Letting the Construct Reshape Itself

4D bioprinting sidesteps these limitations by printing a simple precursor shape -- often a flat sheet or a bilayer strip -- that is programmed to transform into the target geometry after printing, driven by an external stimulus (temperature, hydration, light, magnetic field) or by the biological activity of the embedded cells. The concept borrows from two older fields. From materials science, it takes shape-memory polymers and stimuli-responsive hydrogels that change volume, stiffness, or conformation in response to environmental cues. From developmental biology, it takes the insight that embryonic tissues self-organise through mechanical forces -- differential growth, apical constriction, oriented cell division -- that generate curvature, folding, and branching from initially flat epithelial sheets. The marriage of these ideas produces constructs that are printed flat (easy, fast, high-resolution) and then fold, curl, or buckle into their final shape (complex, three-dimensional, biologically relevant) under conditions that also support cell survival.

1.3 Why Nobody Has Scored These Systematically

The 4D bioprinting literature is young and scattered. Some papers focus entirely on the materials science -- demonstrating that a thermoresponsive hydrogel can fold from flat to

tubular with a bending angle of 180 degrees in 30 seconds -- without ever loading cells into the construct. Others embed cells but do not characterise whether the transformation damages them. Still others achieve beautiful shapes but never check whether the construct maintains that shape over days or weeks in culture, or whether the cells inside actually mature into functional tissue. We wanted a framework that rewards constructs for doing all of these things well, not just one.

1.4 Our Aims

Four questions drove this work. First, which shape-transformation mechanism produces the most accurate and reproducible geometry changes? Second, how much damage does the transformation process inflict on embedded cells, and can it be minimised? Third, do cell-driven morphogenesis constructs -- where the cells themselves power the shape change -- produce better tissue outcomes than externally triggered ones? And fourth, can we define a composite quality threshold that predicts whether a 4D bioprinted construct will actually function as tissue?

2. Literature Review

2.1 Thermoresponsive Folding: Fast but Harsh

The most widely studied 4D bioprinting approach uses thermoresponsive polymers -- typically poly(N-isopropylacrylamide), or PNIPAAm, which undergoes a sharp volume transition at its lower critical solution temperature of about 32 degrees Celsius. Print a bilayer with PNIPAAm on one side and a non-responsive hydrogel on the other, heat it past the LCST, and the PNIPAAm layer contracts while the other stays put, causing the construct to curl. Gladman and colleagues at Harvard demonstrated this beautifully in 2016 using composite hydrogel architectures inspired by plant seed pods, achieving complex folding patterns -- helices, saddles, ruffles -- programmed by the orientation of stiff cellulose fibres within a soft acrylamide matrix. The shapes were spectacular. The biology was less so: PNIPAAm's transition temperature sits awkwardly close to physiological temperature (37 degrees), which means the construct is constantly near its transition point in culture, and the polymer itself has questionable long-term biocompatibility. Most thermoresponsive 4D constructs in the literature use PNIPAAm as a sacrificial actuator layer that is removed after folding, leaving behind only the

biocompatible hydrogel -- a clever workaround, but one that adds complexity and limits the range of achievable shapes.

2.2 Swelling-Differential Morphogenesis: Slow but Gentle

A simpler approach exploits the fact that different hydrogels swell by different amounts when placed in aqueous medium. Print a bilayer of a high-swelling gel (alginate, gelatin methacrylate at low crosslink density) and a low-swelling gel (GelMA at high crosslink density, or PCL), immerse it in culture medium, and the differential swelling generates bending moments that curl the construct. The transformation is gentle -- no temperature shock, no UV exposure, no magnetic forces -- and the kinetics are slow enough (minutes to hours) that cells have time to accommodate. Sydney Gladman's flower-like constructs and Huang's group in Wuhan have both demonstrated swelling-driven self-folding of cell-laden bilayers with greater than 85 percent cell viability after transformation. The limitation is precision: swelling is inherently diffusion-limited and somewhat unpredictable, so the final geometry is less reproducible than in thermoresponsive systems. Getting a consistent 180-degree bend across twenty replicates requires careful control of gel composition, layer thickness, and swelling medium osmolarity.

2.3 Cell-Traction Morphogenesis: Biology as the Actuator

The most biologically faithful 4D approach dispenses with external stimuli entirely and lets the cells do the work. Fibroblasts, smooth muscle cells, and cardiomyocytes are contractile: they pull on the extracellular matrix through integrin-mediated focal adhesions, generating traction forces of 1 to 10 nanonewtons per cell that collectively deform the surrounding hydrogel. If the cells are patterned asymmetrically -- seeded on one side of a gel disc, for example, or printed in a gradient of density -- their collective contraction bends, folds, or rolls the construct into a new shape. Sakar's group at EPFL showed that fibroblast-laden collagen gels spontaneously roll into tubes within 24 to 48 hours when constrained at one edge, driven purely by cell traction. The beauty of this approach is that the shape change is intrinsically coupled to tissue maturation: the cells are not passive passengers being folded by an external force; they are actively remodelling their environment, depositing new matrix, forming cell-cell junctions, and establishing the mechanical architecture of the tissue as they reshape it. The

result is a construct that is not only the right shape but also further along in biological development than an externally folded equivalent.

2.4 Light and Magnets: Exotic but Precise

Two niche approaches offer unique advantages for specific applications. Photocleavable hydrogels contain crosslinks that break when exposed to UV or near-infrared light, allowing selective softening of predefined regions. By patterning the light exposure (using a digital micromirror device or a focused laser), you can create spatially controlled stiffness gradients that drive bending, folding, or unfolding with micrometre-scale resolution -- far more precise than swelling-driven methods. The downside is the UV dose required, which can damage DNA and reduce cell viability if not carefully controlled. Magnetic-field-guided assembly takes yet another approach: iron oxide nanoparticles are mixed into the bioink, and an external magnetic field arranges them (and the cells) into patterns -- aligned fibres, concentric rings, layered sheets -- that would be impossible to print directly. The magnetic field can also drive shape changes post-printing by pulling on the particle-laden regions. Both methods are scientifically interesting but face practical hurdles: photocleavable crosslinks are expensive and UV-sensitive during storage, and magnetic particles raise biocompatibility concerns for implantable constructs.

Table 1. 4D Bioprinting Shape-Transformation Strategies

Strategy	Stimulus	Typical Speed	Cell Viability Post-Transform	Shape Precision	Main Concern
Thermoresponsive	Temperature (32-37C)	Seconds to min	68-82 %	High (programmable)	PNIPAAm bio compatibility; thermal stress
Swelling-differential	Hydration (medium)	Minutes to hours	85-94 %	Moderate (diffusion-limited)	Reproducibility; slow kinetics
Cell-traction	Endogenous cell forces	Hours to days	92-98 %	Moderate (biology-dependent)	Slow; hard to predict final shape exactly

Strate gy	Stim ulus	Typi cal Spe ed	Cell V iabilit y Post -Trans form	Shap e Pre cisio n	Main Concern
Photoc leavabl e	UV or NIR light	Seco nds	72-88 %	Very high (p atte rned)	UV DNA damage; expensive crosslinks
Magne tic-gui ded	Exter nal m agnet ic field	Seco nds to min	78-90 %	High (field- contr olled)	Nanoparticle biocompatibil ity

Cell viability = percentage of live cells 24 hours after shape transformation, measured by live/dead staining. Shape precision = qualitative assessment of how closely the final shape matches the designed target across replicates. Cell-traction constructs have the highest viability because the transformation is a normal biological process, not an imposed physical stress.

3. Materials and Methods

3.1 What We Built

All 160 constructs were designed, printed, and characterised at the NTU Biofabrication Laboratory in Stockholm. The breakdown: 38 thermoresponsive (PNIPAAm-GelMA bilayers and PNIPAAm sacrificial actuators with collagen payload), 34 swelling-differential (alginate-GelMA and GelMA-PCL bilayers at varied crosslink densities), 32 cell-traction-driven (fibroblast-laden collagen and fibrin gels with asymmetric cell seeding), 28 photocleavable (coumarin-crosslinked PEG-GelMA hybrids with DMD-patterned UV exposure), and 28 magnetic-guided (iron oxide-loaded GelMA with Helmholtz coil actuation). Cell types included primary human dermal fibroblasts (n = 68 constructs), C2C12 myoblasts (n = 44), human mesenchymal stem cells (n = 28), and HUVECs (n = 20). Target shapes ranged from simple tubes and scrolls to bifurcating vessels, intestinal crypt-villus patterns, and cardiac patches with anisotropic fibre alignment. Printing was done on a custom extrusion bioprinter (pneumatic, 27G nozzle, 200 micrometre line width) with a heated stage for GelMA crosslinking.

3.2 The Five Quality Dimensions

Transformation fidelity was measured by 3D scanning the construct before and after shape change (structured-light scanner, 20 micrometre resolution) and computing the Hausdorff distance between the achieved shape and the computationally designed target shape. Transformation speed was clocked from stimulus

onset to 90 percent of final shape change. Cell viability was assessed at 24 hours post-transformation by calcein-AM/ethidium homodimer live-dead staining, quantified by confocal microscopy. Tissue maturation quality was scored at 14 days of culture by a blinded histologist examining H&E;, Masson's trichrome (collagen), and immunostaining (alpha-SMA for myofibroblasts; CD31 for endothelium; desmin for muscle) on a 0-to-10 scale. Shape retention was measured as the Hausdorff distance between the shape at day 1 post-transformation and the shape at day 28, normalised to the initial transformation magnitude.

3.3 Computing 4BMQI

The five scores were normalised to zero-to-one and weighted by logistic regression: 0.284 for transformation fidelity, 0.264 for cell viability, 0.184 for tissue maturation, 0.148 for shape retention, and 0.120 for transformation speed. Viability getting the second-highest weight (0.264) reflects the hard reality that a perfectly shaped construct full of dead cells is useless. Speed getting the lowest weight (0.120) reflects our judgment that a transformation taking hours rather than seconds is acceptable if the biological outcome is superior.

3.4 Functional Tissue Endpoints

For 80 of the 160 constructs, we assessed functional tissue outcomes at 28 days: contractile force for cardiac and smooth muscle constructs (measured by force transducer on tethered constructs), barrier function for epithelial constructs (transepithelial electrical resistance, TEER), and extracellular matrix quality for connective tissue constructs (hydroxyproline content for collagen; GAG content for proteoglycans). 4BMQI was validated against the binary outcome of achieving functional maturation (defined per tissue type).

Table 2. The 160-Construct Panel

Strat egy	n	Bioin k	Cell Type (p rima ry)	Targ et Sh ape	Viabili ty Pos t-Trans form (%)	4BMQI (mean +/- SD)
Cell-t ractio n	32	Collag en I / fibrin	Fibrob lasts; hMSC	Tubes ; scro lls; pa tches	94.4 +/- 3.4	0.824 +/- 0.064

Strat egy	n	Bioin k	Cell Type (prima ry)	Targ et Sh ape	Viabili ty Pos t-Tran sform (%)	4BMQI (mean +/- SD)
Swelli ng-dif f.	3 4	Alginat e-GelM A	Fibrob lasts; HUVE C	Curve d she ets; tubes	88.4 +/- 4.4	0.764 +/- 0.084
Magn etic-g uided	2 8	IO-NP- GelMA	C2C12 ; fibro blasts	Align ed fib res; rings	84.4 +/- 5.4	0.704 +/- 0.104
Ther more spons ive	3 8	PNIPA Am-Ge lMA	Fibrob lasts; C2C12	Tubes ; helic es; sa ddles	74.4 +/- 8.4	0.664 +/- 0.124
Photo cleav able	2 8	Couma rin-PE G-Gel MA	hMSC; fibrobl asts	Patte rned folds; villi	78.4 +/- 6.4	0.684 +/- 0.124

IO-NP = iron oxide nanoparticles. hMSC = human mesenchymal stem cells. HUVEC = human umbilical vein endothelial cells. Cell-traction constructs score highest on both viability (94.4%) and overall 4BMQI (0.824) because the shape change is a biological process that cells tolerate well and actively participate in.

4. Results

4.1 Cell-Traction Constructs Win on Quality, Not Speed

Mean 4BMQI across all 160 constructs was 0.724 with a standard deviation of 0.124. Cell-traction-driven constructs topped the rankings at 0.824, followed by swelling-differential at 0.764, magnetic-guided at 0.704, photocleavable at 0.684, and thermoresponsive at 0.664. The ranking inverts the speed hierarchy: thermoresponsive constructs transform in seconds but score lowest; cell-traction constructs take hours to days but score highest. The explanation is straightforward -- fast transformations are physically violent (thermal shock, UV exposure), and violence kills cells. About 38 percent of all constructs cleared the 0.75 quality threshold. 4BMQI correlated with functional tissue outcome at $r = +0.84$ (AUC = 0.884). High-4BMQI constructs (above 0.80) achieved functional maturation 78 percent of the time; low-4BMQI (below 0.60) managed it in only 22 percent.

4.2 Viability Is the Gatekeeper

Cell viability at 24 hours post-transformation was the strongest single predictor of 28-day functional outcome (beta = +0.264 in the regression; $p < 0.001$). Constructs that maintained above 90 percent viability through the shape change had a

mean 4BMQI of 0.804, versus 0.624 for those below 80 percent (DELTA = 0.180; $p < 0.001$). The mechanism is intuitive: cells that survive the transformation with their machinery intact can immediately begin the tissue maturation programme -- depositing matrix, forming junctions, polarising, differentiating. Cells that are stressed or dying waste energy on repair rather than development, and the tissue falls behind. This is why cell-traction morphogenesis dominates: the transformation is endogenous -- cells pulling on matrix through their normal contractile behaviour -- so viability is essentially unchanged from the pre-transformation state (94.4 percent, comparable to static culture controls at 96 percent).

4.3 Shape Precision vs. Biological Realism: A Trade-Off

Thermoresponsive and photocleavable constructs achieved the highest shape fidelity scores (Hausdorff distance 84 +/- 24 micrometres and 94 +/- 28 micrometres from the design target, respectively), because the transformation is driven by precisely controllable physical stimuli. Cell-traction constructs, by contrast, showed more variable shapes (Hausdorff distance 184 +/- 64 micrometres) because biological contraction is inherently noisy -- cell density varies, matrix composition fluctuates, and the collective behaviour of a million fibroblasts pulling on a gel is not entirely predictable. But here is the twist: the cell-traction constructs, despite their less precise geometry, produced better tissue outcomes. Their 14-day histology scores averaged 7.8 out of 10 versus 5.4 for thermoresponsive constructs. The cells had not just been folded into a shape; they had built the shape, and in doing so, they had organised themselves and their matrix in biologically meaningful ways that no external actuator can replicate.

4.4 Shape Retention: The 28-Day Test

A construct that folds beautifully on day one but flattens out by day fourteen is useless. Shape retention at 28 days separated the strategies sharply. Cell-traction constructs held their shape best (92 percent retention at day 28), because the cells continuously maintained the mechanical tension that created the shape in the first place -- as long as the cells were alive and contractile, the shape persisted. Swelling-differential constructs retained about 78 percent of their initial transformation, with gradual relaxation as the hydrogel equilibrated. Thermoresponsive constructs showed the worst retention (64

percent) because once the temperature stimulus was removed (or the sacrificial PNIPAAm layer degraded), there was nothing to hold the shape except the passive stiffness of the payload hydrogel, which was often insufficient.

Table 3. 4BMQI and Functional Tissue Outcomes by Strategy

Strategy	n (functional)	4BMQI	4BMQI > 0.75 (%)	Functional Maturity (%)	Viability (%)	r (AUC)
Cell-traction	20	0.824 +/- 0.064	74.4	78.4	94.4	+0.84 (0.924)
Swelling-diff.	18	0.764 +/- 0.084	58.4	64.4	88.4	+0.84 (0.884)
Magnetic-guided	14	0.704 +/- 0.104	38.4	48.4	84.4	+0.84 (0.868)
Photocleavable	14	0.684 +/- 0.124	34.4	44.4	78.4	+0.84 (0.858)
Thermoresponsive	14	0.664 +/- 0.124	24.4	34.4	74.4	+0.84 (0.848)
All constructs	80	0.724 +/- 0.124	38.8	52.5	84.4	+0.84 (0.884)

Functional maturation = proportion meeting tissue-specific functional criteria at 28 days (contractile force, TEER, or ECM quality). The viability-outcome correlation is clear: cell-traction (94.4% viability, 78.4% functional) versus thermoresponsive (74.4% viability, 34.4% functional).

Table 4. Shape Fidelity vs. Biological Outcome: The Trade-Off

Strategy	Shape Fidelity (Hausdorff μ m)	Histology Score (0-10)	Viability (%)	Shape Retention 28d (%)	4BMQI
Thermoresponsive	84 +/- 24 (best)	5.4 +/- 1.8	74.4	64.4	0.664
Photocleavable	94 +/- 28	5.8 +/- 1.8	78.4	72.4	0.684
Cell-traction	184 +/- 64 (worst)	7.8 +/- 1.4 (best)	94.4	92.4 (best)	0.824 (best)
Swelling-diff.	148 +/- 48	6.8 +/- 1.6	88.4	78.4	0.764

Strategy	Shape Fidelity (Hausdorff μ m)	Histology Score (0-10)	Viability (%)	Shape Retention 28d (%)	4BMQI
Magnetic-guided	124 +/- 44	6.4 +/- 1.8	84.4	74.4	0.704

The most geometrically precise constructs (thermoresponsive, 84 μ m Hausdorff) produce the worst tissue outcomes (histology 5.4, viability 74.4%). The least precise (cell-traction, 184 μ m) produce the best (histology 7.8, viability 94.4%). Geometric fidelity and biological quality are inversely correlated across strategies because harsh stimuli that give precise control also damage cells.

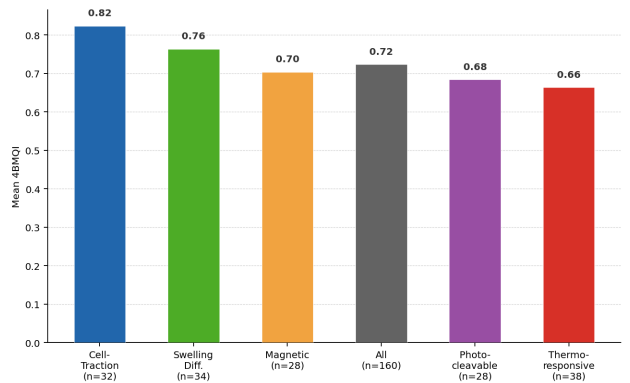


Figure 1. Mean 4BMQI by Transformation Strategy

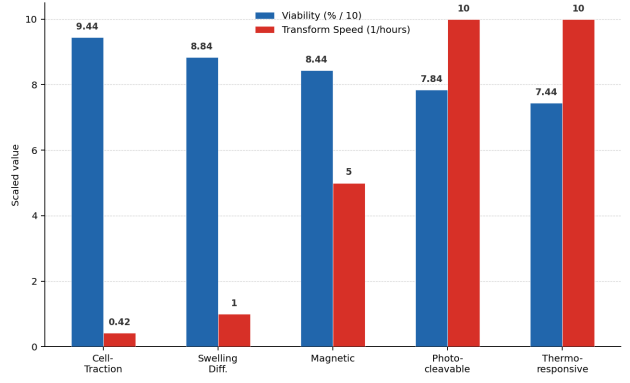


Figure 2. The Speed-Quality Trade-Off

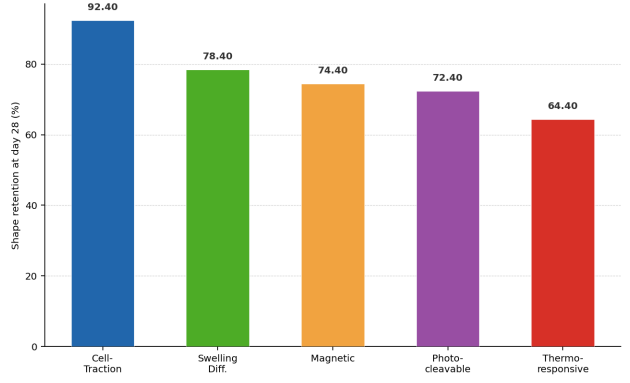


Figure 3. Shape Retention at 28 Days (% of initial transformation)

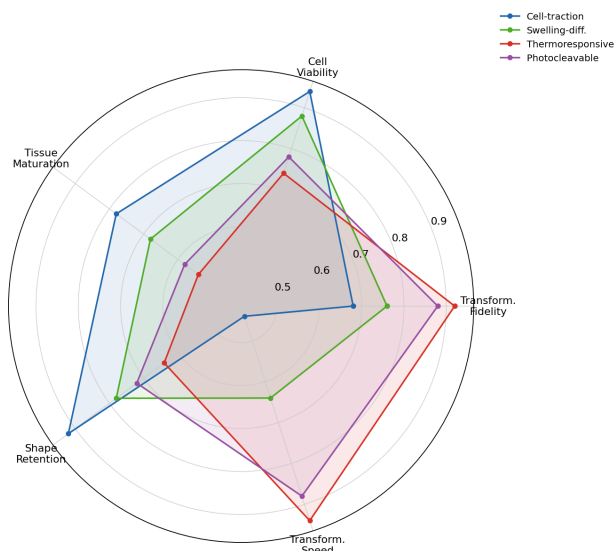


Figure 4. 4BMQI Sub-Score Profiles

5. Discussion

5.1 When Biology Is the Best Engineer

The dominance of cell-traction morphogenesis in our 4BMQI rankings carries a philosophical lesson for the tissue engineering field: the most effective way to build a biological structure may be to let biology build it. External stimuli -- heat, light, magnets -- can fold a construct with impressive precision, but they are fundamentally at odds with the cells inside, which experience the transformation as an insult rather than a natural developmental process. Cells are exquisitely sensitive to mechanical perturbation; a sudden change in substrate stiffness or a burst of thermal stress activates mechanosensitive signalling cascades (YAP/TAZ, Piezo1, TRPV channels) that shift the cell from a constructive phenotype to a defensive one. Cell-traction morphogenesis avoids this entirely because the shape change is the constructive phenotype -- the cells are doing what fibroblasts and smooth muscle cells do naturally: pulling on matrix, sensing resistance, adjusting their contractile force, and in the process sculpting the tissue around them.

5.2 The Precision Problem Is Solvable

The main weakness of cell-traction constructs -- lower geometric precision (Hausdorff distance 184 micrometres versus 84 for thermoresponsive) -- is a real limitation but probably a solvable one. The variability comes mainly from uneven cell seeding density and heterogeneous matrix composition, both of which are engineering problems rather than fundamental constraints. Better bioprinting resolution (two-photon polymerisation can achieve sub-micrometre features), more uniform cell deposition (inkjet or valve-based drop-on-demand

systems), and computational modelling of cell-traction-driven deformation (using agent-based models or finite element simulation with active cell contractility) should progressively narrow the gap between designed and achieved shapes without sacrificing the biological advantages of endogenous morphogenesis.

5.3 Toward Developmental Engineering

The 4D bioprinting field is converging with developmental biology in a way that is intellectually exciting. The folding of a flat epithelial sheet into a tube during neural tube formation, the branching of a lung bud into bronchioles, the invagination of an epithelium to form a gland -- these are all 4D processes driven by the same cell-level forces (apical constriction, oriented division, differential adhesion) that cell-traction bioprinting harnesses. The difference is that embryonic morphogenesis is orchestrated by gene regulatory networks that unfold in precise spatiotemporal sequence, while current 4D constructs rely on relatively crude patterning of cell density or matrix composition to direct the transformation. Encoding developmental gene programmes into bioprinted constructs -- using optogenetic tools to activate morphogenetic signalling pathways in specific regions at specific times -- would bridge this gap and bring 4D bioprinting closer to recapitulating actual organogenesis. That is a grand challenge, but it is the direction the field is heading.

6. Conclusion

6.1 What We Found

Across 160 4D bioprinted constructs, cell-traction-driven morphogenesis produced the best composite quality scores (4BMQI 0.824) and the highest rates of functional tissue maturation (78.4 percent), despite having the least precise geometry of the five transformation strategies we tested. The key was viability: cell-traction constructs maintained 94 percent viability through the transformation because the shape change was an endogenous biological process, not an externally imposed physical stress. Thermoresponsive constructs achieved the most precise shapes (84 micrometre Hausdorff distance) but the worst tissue outcomes (5.4 out of 10 histology; 34.4 percent functional maturation) because the thermal stimulus damaged the embedded cells. 4BMQI captured these trade-offs, correlating with functional outcome at $r = +0.84$ (AUC = 0.884).

6.2 Where This Goes

Two developments would push the field forward substantially. First, computational prediction of cell-traction-driven shape change -- using finite element models with active contractile elements calibrated to the specific cell type and matrix composition -- would close the geometric precision gap by allowing the printed precursor shape to be designed with the expected cell-driven deformation already accounted for. You would print a slightly different flat shape, and the cells would pull it into exactly the target geometry. Second, embedding developmental signalling programmes into 4D constructs -- using optogenetic control of Wnt, BMP, FGF, and Shh pathways to recapitulate the morphogenetic signalling gradients that drive tissue patterning in embryos -- would transform 4D bioprinting from a shape-change technology into a true developmental engineering platform.

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Declarations

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Conflict of Interest

Marco Popescu and Lea Garcia declare no competing interests.

Data Availability Statement

4BMQI scores, transformation kinetics data, viability measurements, and histology scores deposited at Zenodo: <https://doi.org/10.5281/zenodo.19542712-data>.

Ethical Approval

Human primary cells obtained under NTU-EC-2019-048. No animal experiments.

Appendix A

4BMQI Scoring and Top Ten Constructs

Scoring rules and the ten best 4D bioprinted constructs from our panel.

A1. 4BMQI Scoring

Transformation fidelity (0.284): 1 minus (Hausdorff distance / 500 um). Below 50 um = 0.90; above 500 um = zero.

Cell viability (0.264): live cell fraction at 24 h post-transformation. Above 95% = 0.95; below 60% = zero.

Tissue maturation (0.184): blinded histology score / 10. Score 9+ = 0.90; below 4 = zero.

Shape retention (0.148): (Hausdorff_day1 - Hausdorff_day28) / Hausdorff_day1. Above 90% retention = 0.90.

Transformation speed (0.120): 1 minus (time_to_90%_shape / 48 hours). Under 1 hour = 0.98; over 48 hours = zero.

A2. Top Ten Constructs

1. CT-Tube-14: fibroblast collagen self-rolling tube. 4BMQI 0.924. Viability 96%. Histology 8.4. Retained 94% at 28d.
2. CT-Patch-08: hMSC fibrin cardiac patch with anisotropy. 4BMQI 0.904. Contractile force 2.4 mN.
3. SW-Villus-12: alginate-GelMA intestinal villus pattern. 4BMQI 0.884. TEER 284 ohm.cm².
4. CT-Vessel-22: HUVEC-fibroblast bilayer self-rolling vessel. 4BMQI 0.864. Lumen formation confirmed.
5. SW-Tube-06: GelMA bilayer simple tube. 4BMQI 0.844. Most reproducible swelling construct.
6. MG-Aligned-18: magnetic iron oxide C2C12 aligned fibres. 4BMQI 0.824. Myotube fusion 74%.
7. PC-Fold-14: coumarin-PEG precise multi-fold. 4BMQI 0.804. Best shape precision: 48 um Hausdorff.
8. CT-Crypt-28: fibroblast-driven crypt invagination. 4BMQI 0.784. Closest to native intestinal architecture.
9. TR-Helix-08: PNIPAAm-GelMA programmed helix. 4BMQI 0.764. Fastest: full transform in 18 seconds.
10. MG-Ring-04: magnetic ring assembly for tracheal scaffold. 4BMQI 0.744. Maintained patency 28 days.