Cybersecurity Research in 3D Bioprinting: Experiments and Evaluation Challenges and Future Directions

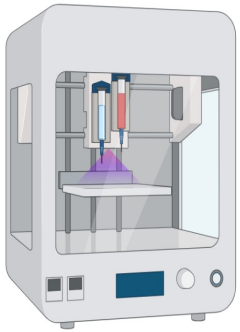
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LASER WORKSHOP ACSAC 2024, HI, USA
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Introduction

```
G0 Z0 F4800.0 ; move to first layer
G0 X4.0 Y-4.0 F4800.0 ; move to beginning
M760
G4 P100 ; dwell for 100 milliseconds
G1 X4.0 Y4.0 F600
G4 P100 ; dwell
G1 X-4.0 Y4.0
G4 P100 ; dwell
G1 X-4.0 Y-4.0
G4 P100 ; dwell
G1 X4.0 Y-4.0
M761
;; layer one complete.
```



What is 3D Bioprinting?

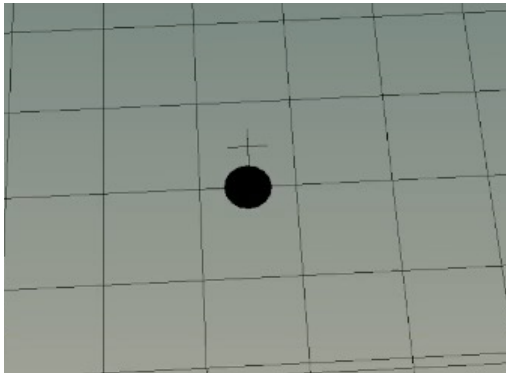
- Application of Additive Manufacturing
- Layered Deposition of material
- Precision in creating complex geometries

Legacy methods

- Manual Seeding
- Mold based

Application of 3D Bioprinting

- Tissue Engineering: Scaffold fabrication
- Organ Transplant: Pre-surgical planning
- Disease Modeling: Cancer research, drug testing
- Regenerative Medicine: Skin grafting

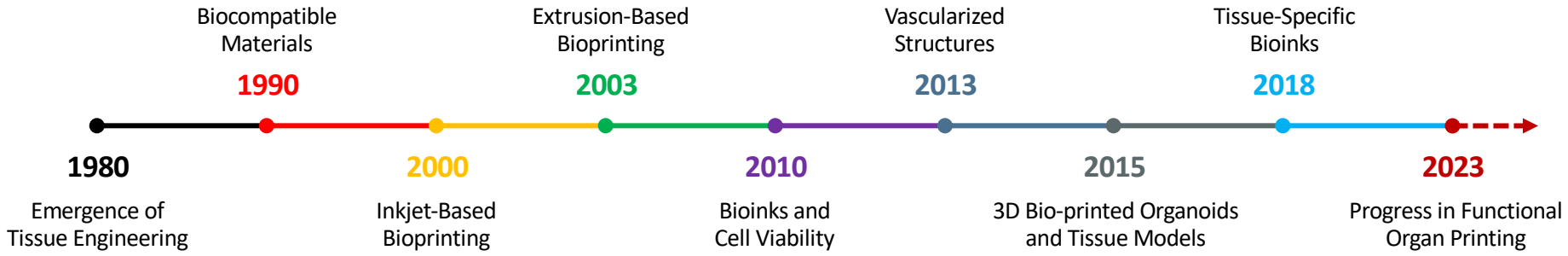


Motivation

Technology is in early development stages

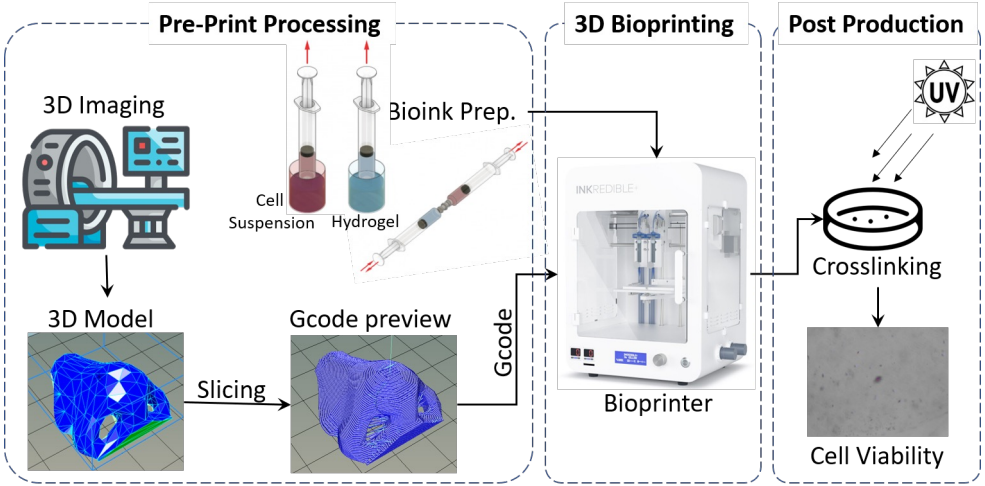
No artifacts available for adversarial attacks

No security framework available



Extrusion Based Bioprinting (EBB)

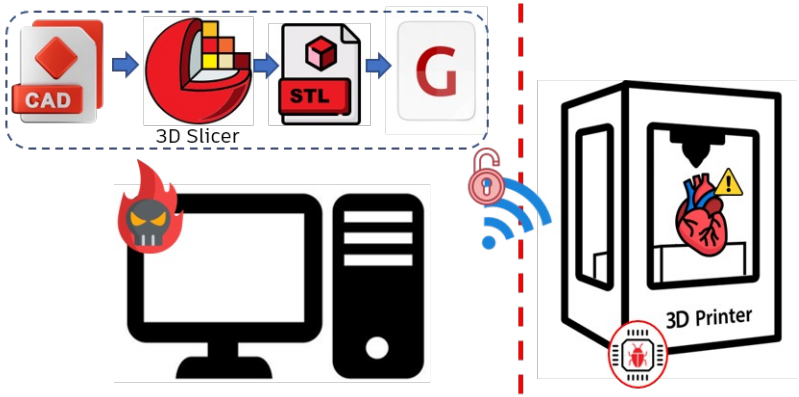
1. Pre-print
   - 3D Imaging, Modeling, Slicing
   - Bioink preparation
2. Bioprinting
   - Layered Material deposition
3. Post-production
   - Crosslinking
   - QA assurance



Adversarial Model

Attack target
- CAD file
- G-code file
- Slicing parameters
- Firmware configurations

Attack vector
- Unencrypted Communication channels
- Supply chain – Malicious firmware update
- Vulnerabilities in Slicing software



Sabotage Attacks

Attacks targeting the print construct

Adverse changes in printing parameters such as

- Thermal settings
- Material placement
- Dimensions
- Filter fan speed

Resulting in non-conformance to user design specifications

QA Parameters

Printability Ratio: Dimensionless method of defining bioink printability.

- Pr < 1 less degree of gelation
- Pr > 1 larger degree of gelation.
- Pr = 1 perfect square shape

$$Pr = L^2/16A$$

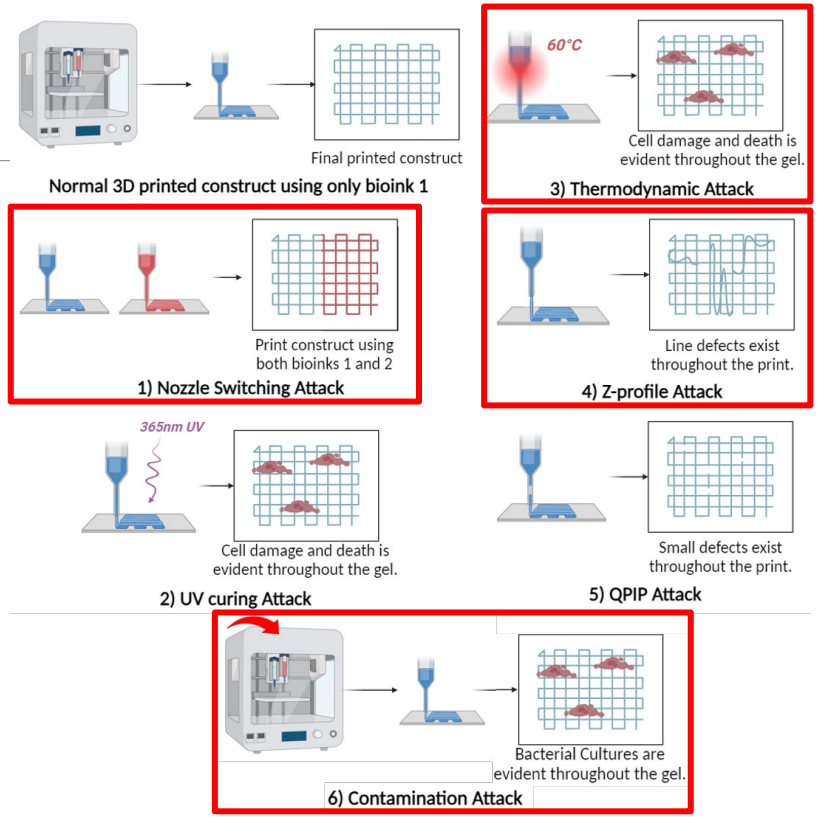
Parameter Optimization Index (POI): Uses nozzle diameter, extrusion pressure, and filament width to determine how well a geometry can be printed with higher accuracy

$$POI = 1/W_a * E_p * N_d$$

Cell Viability: Percentage of live cells after printing.

Proposed Attacks

1. Nozzle Switching
2. UV Curing
3. Thermodynamic
4. Z-profile
5. Quick pause in print (QPIP)
6. Contamination



BioSaFe – Security Framework

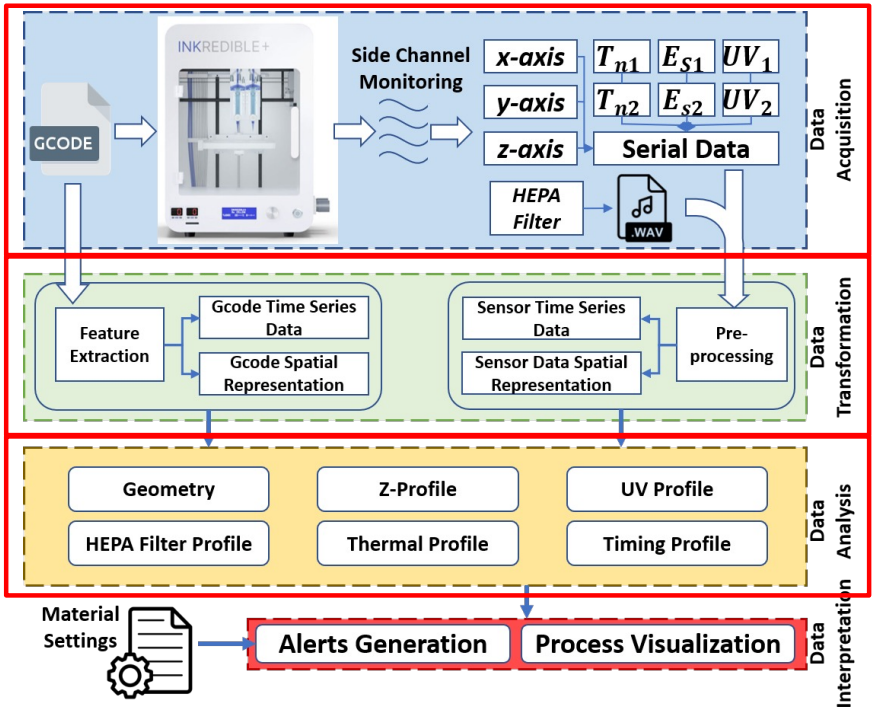
Objectives

1. In-situ physical process monitoring
2. Supports bioprinting specific needs
3. Requires no training data
4. Supports mass customization of print geometry
5. Low cost and adaptable

BioSaFe – Framework Architecture

Four Layer architecture

1. Data Acquisition
2. Data Transformation
3. Data Analysis
4. Data Interpretation



Sensor Selection

Intrusiveness

- Sensors should be deployed with minimal retrofitting and without affecting the physical process

Calibration Complexity

- Sensors should be easily calibrated to make the framework scalable.

Noise Sensitivity

- The sensor data should be less sensitive to environmental noise to provide more accurate physical process information.

Data Acquisition

G-Code for ground truth and sensing data for physical monitoring

Frequent Data (5ms)

- Nozzle Position (x, y)
- Extruder State

Non-frequent changes (50ms)

- Nozzle Temperature
- Bed Position

UV intensity (5 sec)

- HEPA Filter fan speed

Data Transformation – Time series

Input

- Initial nozzle coordinates
- Final nozzle coordinates
- Speed
- Maximum acceleration

Output

- Nozzle Position for each instance of time between the given coordinates.

Algorithm 1 Time Domain Transformation Function

Input: $NP_{x1}, NP_{y1}, NP_{x2}, NP_{y2}, v_{max}, a_{max}$

$x_1 \leftarrow NP_{x1}$, $x_2 \leftarrow NP_{x2}$, $y_1 \leftarrow NP_{y1}$, $y_2 \leftarrow NP_{y2}$
$D \leftarrow \sqrt{(x_2 - x_1)^2 - (y_2 - y_1)^2}$
$\theta \leftarrow tan^{-1}((y_2 - y_1)/(x_2 - x_1))$
$S \leftarrow \sqrt{D * a_{max}}$
if $S < v_{max}$ **then** ▷ Triangular motion
  $T \leftarrow 2\sqrt{D/a_{max}}$
  $t \leftarrow T/2$, $d \leftarrow a_{max}t^2/2$
  while $temp < T$ **do**
    $t_i \leftarrow temp * \Delta t$
    if $temp < t_i$ **then** ▷ Acceleration phase
      $\Delta d \leftarrow 0.5 * a_{max} * t_i^2$
    else ▷ Deceleration phase
      $\Delta d \leftarrow d + S * t_i - 0.5 * a_{max} * t_i^2$
    end if
    $intp_x \leftarrow \Delta d cos\theta$
    $intp_y \leftarrow \Delta d|sin\theta|$
    $TSD_array \leftarrow insert(t_i, intp_x, intp_y)$
  end while
end if
return TSD_array

Data Transformation – Space Domain

Input
- Initial nozzle coordinates
- Final nozzle coordinates
- Extruder status

Output
- Per layer line plot

Algorithm 2 Space Domain Transformation Function

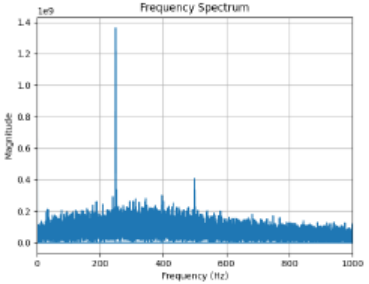
Input: $NP_{x1}, NP_{y1}, NP_{x2}, NP_{y2}, ES$
Output: plot_{layerNum}.png

$x_1 \leftarrow NP_{x1}$, $x_2 \leftarrow NP_{x2}$, $y_1 \leftarrow NP_{y1}$, $y_2 \leftarrow NP_{y2}$
Open new Plot
if ES_1 is high **then** ▷ Nozzle 1 is active
    $Color \leftarrow$ **black**
    Plot black-line using the coordinates
else if ES_2 is high **then** ▷ Nozzle 2 is active
    $Color \leftarrow$ **blue**
    Plot blue-line using the coordinates
else
    $Color \leftarrow$ **red** ▷ No extrusion
    Plot red-line using the coordinates
end if
if $LC_i == TRUE$ **then** ▷ Layer change event
    Save **plot_{layerNum}.png**
    Close current plot
    Open a new plot
end if

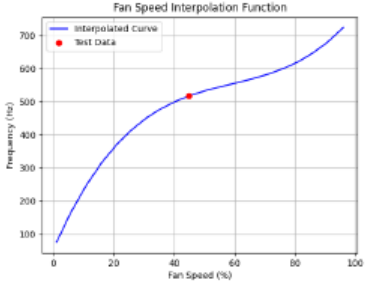
Data Transformation – HEPA Filter

- Noise to frequency domain
- High amplitude corresponds to fan noise.
- Train an cubic polynomial mapping function to estimate fan speed.

$$fr_{spec} = fft(audio_file.wav)$$
$$fr_p = max(|fr_{spec}|)$$
$$FS = f^3(fr_p)$$



(a) Fan speed is 10%



(b) Estimator func.

Fig. 5: Frequency spectrum and interpolation curve of the captured fan noise.

Data Analysis

The module performs verification of the following 6 profiles

1. **Geometry Profile** – Per-layer geometry verification, encompassing both the infill and outer structure.
2. **Z-Profile** – Layer Change Event Array
3. **Timing Profile** – $[\![TP]\!]_gcode$ and $[\![TP]\!]_sensor$ synchronized and verified
4. **Thermal Profile** – Compensate errors due to time to require thermal equilibrium
5. **UV Profile** – Duration, distance from the source and type of UV
6. **Fan Speed Profile** – To verify HEPA filter performance/Speed

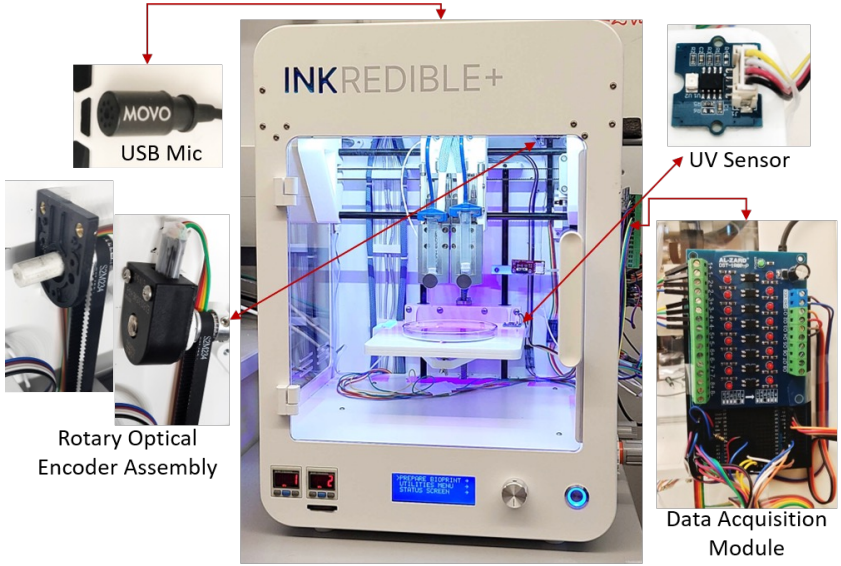
Experimental Setup

Inkredible+ 3D Bioprinter

- Dual Nozzle
- HEPA filtration unit
- Temperature control
- UV curing
- Pneumatic extrusion

Sensors

- Microphone – Fan speed
- Optical rotary encoder – x,y,z position
- Thermistors – Nozzle temperature
- UV sensor – UV type



Installation and Calibration

Rotary optical encoder

- Placement - Extending motor pulleys to support installation of through hole encoder
- Convert rotational movement to linear movement

Microphone

- Placement - Close to HEPA filter
- Convert noise data to fan speed

UV Dose

- Placement - Under UV light on print bed
- Using standoff distance, time, and intensity to measure UV Dose

Thermistor

- Placement - On the metallic part of the cartridge holder
- Using time window to allow for thermal equilibrium

Hydrogel Medium

- Supports cell growth, and proliferation.
- Encapsulate living cells, keeping them hydrated and protected.
- Are biocompatible, ensuring that they do not harm the cells.
- Allows for nutrient diffusion and waste removal, essential for cell survival.
- Biodegradable over time.
- Provides structural integrity.

Hydrogel Preparation

Prepare Agarose Solution:

- Dissolve 2% (w/v) agarose in Complete Dulbecco's Modified Eagle Medium (DMEM + 5% Fetal Bovine Serum + 1% Pen-Strep).
- Heat at 80°C for 1 hour, stirring continuously.

Prepare Gelatin Solution:

- Dissolve 2% (w/v) gelatin in Complete DMEM.
- Heat at 55°C for 1 hour, stirring continuously.

Combine Solutions:

- Mix the heated agarose and gelatin solutions in a beaker.
- Maintain 55°C and stir for 1 hour to achieve homogeneity.

Transfer Hydrogel:

- Extract the prepared hydrogel with a sterile syringe.
- Transfer to a bioprinting cartridge, removing air bubbles.

Bioink Formulation

Cell Culture

- A549 cells were cultured in Complete DMEM.
- Cells were maintained under incubation conditions of 37°C with 5% CO_2.
- A549 cells have a doubling time of 24 hours and were fed daily with fresh media.

Cell Harvesting

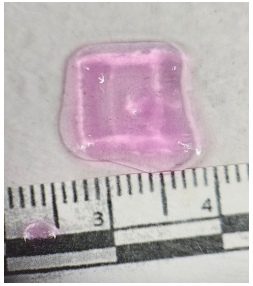
- At a 80% confluency, cells were split and counted.
- A pellet was then created with the desired quantity for the bio mixing procedure.

Mixing with Hydrogel

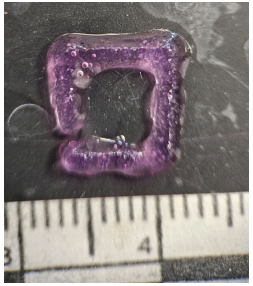
- Suspend the cell pellet in 0.2 mL sterile media and transfer to a 3 mL syringe.
- Connect to a 2 mL prewarmed hydrogel-filled cartridge via a syringe coupler.
- Push and pull the suspension 3 - 5 times to mix evenly, avoiding bubbles.

Challenges with Bioink

- Contamination of cell culture.
- Air bubbles trapped in bioink.
- Maintaining the optimal temperature.
- Maintaining cell viability during bioink preparation.
- Accidents – Cell storage equipment breakdown.



Unoptimized temperature setting resulting in poor gelation



Air bubble resulting in structural compromise



Air bubble in the cartridge during bioink transfer

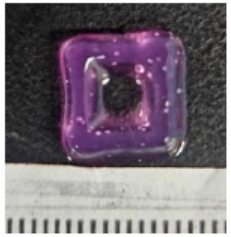
Attacks Evaluation

Print Constructs
- Square shape – Help measure Pr and POI
- Ear shape – Complex geometry case study

Live Cells
- Lung cell A549

Hydrogel
- Gelatin-agarose gel

Bioink
- A549 cells at a concentration of 3 million cells per mL of hydrogel



Square Construct



Ear Shape construct

Cell viability

Staining

- Stain cells with NucBlue (all cells) and propidium iodide (dead cells).
- Incubate for 15 mins, then aspirate the colored media and replace with fresh media.

Imaging

- Capture images at four locations per construct: top, right, bottom, and left, using a Cytation Plate Reader.
- Count total cells (EX377-EM447) and dead cells (EX586-EM647).

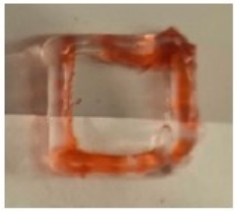
Viability Calculation

- Calculate live cells as: Live Cell Count = Total Cell Count − Dead Cell Count
- Determine percentage viability: Viability (%) = (Live Cell Count / Total Cell Count) × 100
- Compute average viability across multiple constructs.



Attack Results – Nozzle Switching

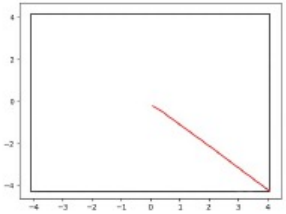
- Changing the material composition
- Two hydrogels, one is dyed with orange color
- Visual representation
- Layer-2 material is switched with Type-2 hydrogel



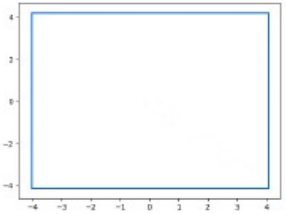
(a) Attack construct (top view)



(b) Attack construct (side view)



(c) Layer-1 of constuct



(d) Layer-2 of constuct

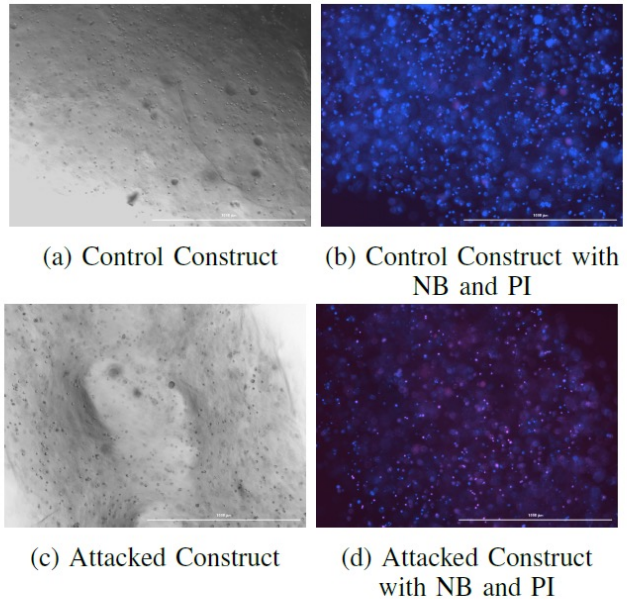
Fig. 6: Nozzle Switching attack with spatiotemporal view.

Attack Results – Thermal

- Normal Construct – at 37°C
- Attack Construct – at 60°C
- Equipment – BioTek Cytation 3: Cell Imaging Multi-Mode Reader

Day #	Print Construct	DAPI count (Total)	TR (Dead)	Live cells	% viability
Control Day 0	Construct 1	1153	34	1119	97
	Construct 2	589	8	581	98.6
Control Day 1	Construct 1	3235	402	2833	87.6
	Construct 2	3539	344	3195	90.3
Attack Day 0	Construct 1	2796	52	2744	98.1
	Construct 2	2533	37	2496	98.5
Attack Day 1	Construct 1	1997	562	1435	71.8
	Construct 2	2780	993	1787	64.3

TABLE IV: Cell viability of control (cells held at 37°C) and temperature attack (cells held at 60°C) print constructs.



(a) Control Construct (b) Control Construct with NB and PI

(c) Attacked Construct (d) Attacked Construct with NB and PI

Fig. 7: Cell viability after 24 hrs for normal and attacked construct

Attack Results – Z profile

Three attack magnitudes with increasing distance from print bed

- 0.5mm, 1.0mm, and 1.5mm

Corresponding measured Pr values

- 0.93, 0.87, and 1.2

The Pr for 1.5mm was found to be farthest from 1, indicating more deviation from the ideal square shape.

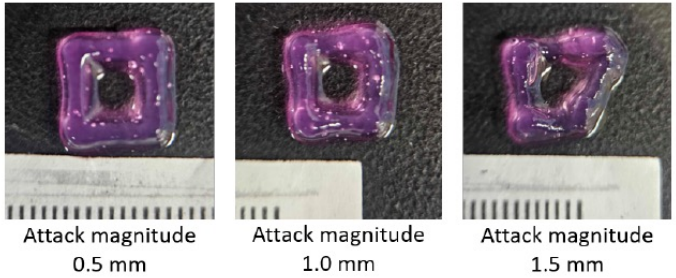


Fig. 8: Z-profile attack on square construct.

Attack Results – Infill Geometry

- Comparing the G-Code with sensor data
- Compared using the geometric verification algorithm
- Any pauses in extrusion could be identified per layer basis

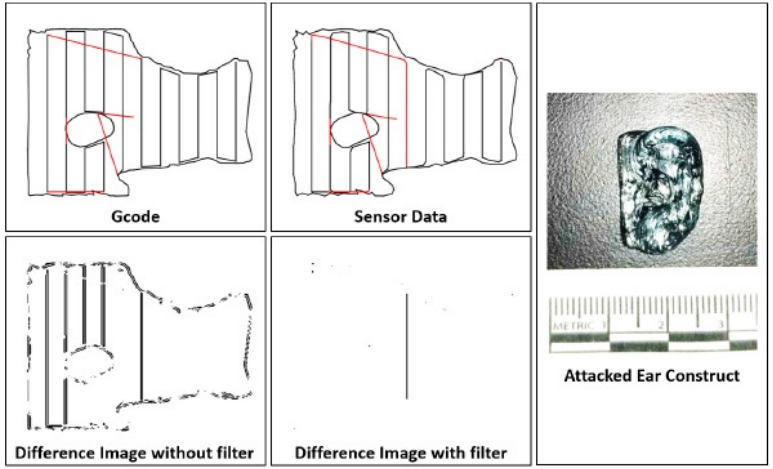
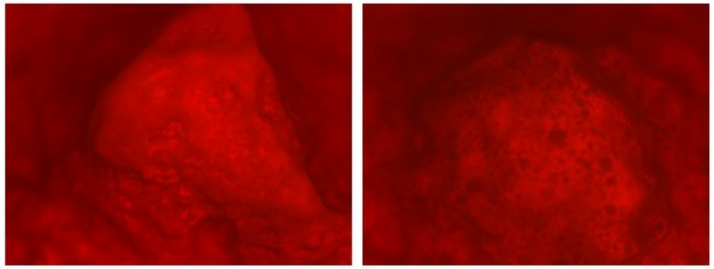


Fig. 11: Infill attack on human ear construct Layer 1.

Attack Results – Contamination

- Hydrogel without embedded cells
- Print exposed to pathogens with HEPA filter off
- Bacterial colonies shown as dark spots
- Incubated for 2 weeks



(a) Normal Construct (b) Contaminated Construct

Fig. 10: Contamination attack on ear construct, examined under a microscope with 3X zoom.

UV Curing – Unexpected Results

- Expected Results – Decreased cell viability due to prolonged UV exposure
- On day 0 the cell viability reduced from 97% to 85%, however, 24 hrs post-printing, it increased back to 94%
- Suggest cell recovery from UV damage or cell proliferation within the hydrogel.
- Replicating the attacks with two cell types

BioSaFe – Evaluation Results

Detection types -> Alert and Visual

Detection Performance -> Current layer or start of next layer

Sr. No.	Attacks	Verifying Profile	Attack Magnitude	Detection Performance	Detection type
1	Nozzle Switching	Geometry	$1mm$	Next layer	Visual
2	UV	UV Profile	UV type, $\Delta t > 1sec$ $\Delta z > 1mm$	Current layer	Alert
3	Nozzle Temp.	Thermal	$3°C$	$\Delta T > 2°C$	Alert
4	Layer Thickness	Z-Profile	$0.1mm$	Next layer	Alert
5	QPIP (Outer)	Geometry	$1mm$	Next layer	Visual
6	QPIP (Infill)	Geometry	$1mm$	Next layer	Visual
7	HEPA filter	Fan Speed	$\pm 2\%$	$5sec$	Alert
8	Print Speed	Timing	$\Delta S = 100mm/sec$	$\Delta t > 2sec$	Alert

TABLE V: BioSaFe attack detection performance.

Interdisciplinary Team

COMPUTER SCIENCE

Attack Simulation and Mitigation
- Identify potential attack vectors.
- Simulate adversarial manipulations in a controlled environment.

Framework Design
- Develop and implement defense mechanisms.
- Build a system for in-situ monitoring and anomaly detection.

Sensor Integration
- Configure and calibrate sensors to collect real-time data.
- Synchronize sensors with bioprinting workflows.

Data Handling
- Collect, process, and analyze sensor data.
- Extract meaningful insights from time-series data and G-code logs.

BIOMEDICAL SCIENCE

Bioink and Hydrogel Preparation
- Develop and refine bioink formulations, including hydrogels suitable for live cells.

Cell and Material Interactions
- Evaluate how changing print parameters impacts cell behavior and survival.
- Assess post-print structural integrity.

Cell Health and Viability
- Perform cell viability testing post-print and after incubation.
- Conduct proliferation assays to evaluate long-term cellular health.

How did we make it work?

Interdisciplinary knowledge gaps

- Conduct Workshops
- Train teams to understand basic concepts

Work frequency

- Understanding the experiment needs
- Effectively communicate the process timeline
- Come up with deadlines with mutual consensus

Artifact validation across discipline e.g, What magnitude is considered as an attack?

- Dual validation
  - Evaluate attack artifact for detectability by cyber security experts
  - Evaluate the attack impact on the print construct by biomedical team

Cont …

Communication barrier e.g, terminologies, methodologies, etc.

- Have frequent meetings
- Create shared resources/repositories

Misaligned priorities

- Have clear understanding of project goals
- Sharing the progress across team

Team Evolution

- Documentation
- Trainings
- Backup data

Reproducibility

Monitoring framework
- Using over the shelf sensors
- Generic algorithms for data interpretation

Data variability due to operating conditions
- Work in controlled environments
- Using sensors more tolerant to environmental conditions

Inconsistent results across different runs (QA aspect)
- Following same protocol for bioink preparation
- Documenting the bioink preparation steps

Scalability

- Minimum retrofitting required
- No need for per-setup training
- More applicable in an industry 4.0 environment with custom designs
- Adaptable to work with other bioprinting technologies

Technology type	Bioink dispensing	BioSaFe monitored profiles						Potential sensing adaptations for BioSaFe
		Gm	LT	UV	HF	Th	PS	
Extrusion based [74]	Piston, Screw, Pneumatic	✓	✓	✓*	✓*	✓*	✓	Choose sensors based on extrusion type
Inkjet [75]	Piezoelectric, Thermal	✓	✓	-	✓*	✓*	✓	Integration of techniques to monitor the droplets
Laser induced [76]	Photothermal energy	✓	-	-	✓*	-	-	Laser parameters such as pulse width, duration, and frequency monitoring
Stereolitho-graphy [77]	Photothermal energy	✓	✓	✓	✓*	-	-	Calibrate UV sensors for different photopolymer sensitivities

* Gm: Geometry, LT: Layer Thickness, HF: HEPA Filter, Th: Thermal, PS: Print Speed

Conclusion

Proposed novel sabotage attacks targeting the printability and cell viability.

Proposed a framework that provides

- In-situ process monitoring
- Doesn’t require training data
- Adaptable to changing printing needs
- Minimally intrusive

Evaluation was done on A549 human lungs cells.

Framework was able to successfully detect printing process variations

Meta-Discussion

- Did you use any experimentation artifacts from the community? No
- Did you attempt to replicate or reproduce earlier research? No
- What insights can be gained from your methodology?
- What challenges did you face, and how did you overcome them?
- Did you produce any intermediate or unsuccessful results? Yes
- Have you shared your experimental artifacts with the community? Not Yet (Planning)

Thanks for your attention!

Questions?

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Commonwealth Cyber Initiative