3D PRINTING IN DRUG DELIVERY AND TISSUE ENGINEERING TECHNOLOGIES

LISA DU TOIT

ASSOCIATE PROFESSOR OF PHARMACEUTICAL CHEMISTRY
SENIOR SCIENTIST: WITS ADVANCED DRUG DELIVERY PLATFORM RESEARCH UNIT

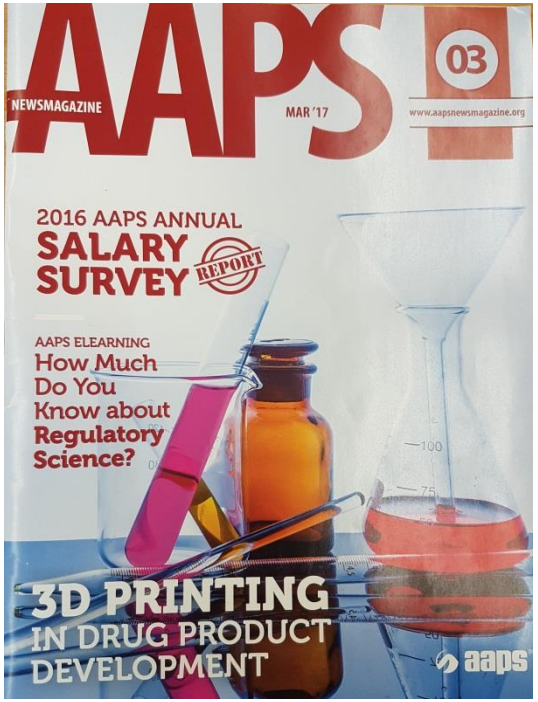
DEPARTMENT OF PHARMACY AND PHARMACOLOGY, WITS UNIVERSITY

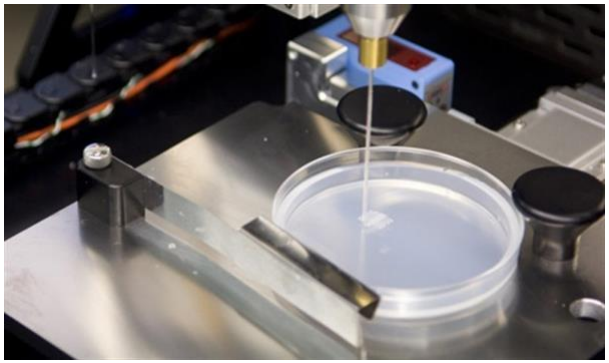
WADDP
WITS Advanced Drug Delivery Platform

SAAPI
South African Association of Pharmacists in Industry

INTRODUCTION

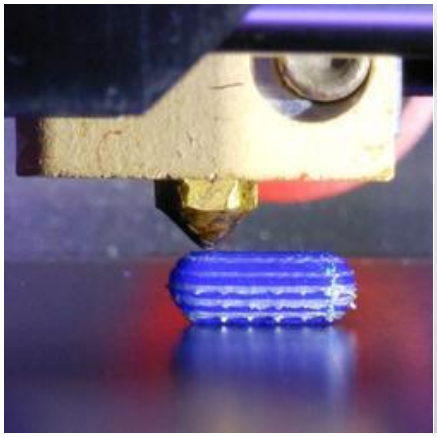
- 3D-Printing = a highly futuristic technology until recently.
- Was predominantly used in manufacturing as stereolithography - create almost any object by fusing different materials - layer by layer → physical version of a digital 3D image.
- Over the past 15 years - 3D printing expanded into healthcare industry.
- Fast becoming established as an advanced and transformative manufacturing technique:
  - creating a solid 3D object from a digital model
  - on demand
  - at a relatively low cost
  - customisable, complex shapes, surfaces and architectures
  - diverse materials
- The convenient cost-effective manufacture of personalised pharmaceutical, medical and dental products = ↑↑ advantage over traditional manufacturing methods.
- Opportunity to use it for personalized healthcare.

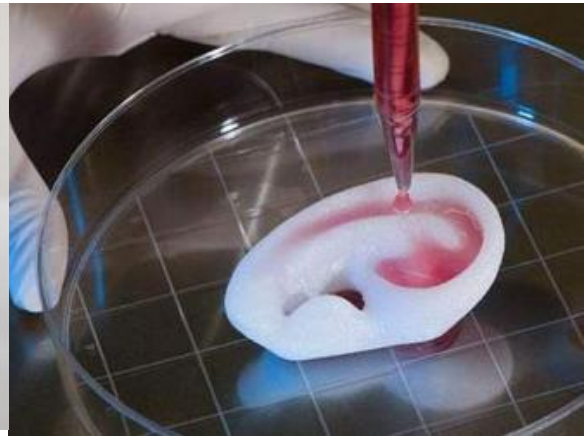




APPLICATIONS

- Pharmaceutical drug research and development could be improved drastically by 3D printing – cheaper and safer drug testing
- Application of 3D printing to medicine - revolutionary implications - enhancing the quality of patients' lives.
- Actual and potential applications include:
  - Customizable 3D-Printed Drugs, Drug Delivery Devices and Implants
  - Medical Prostheses
  - Anatomical Models for Surgical Preparation and Surgical Practice
  - Bioprinting of Tissues and Organs



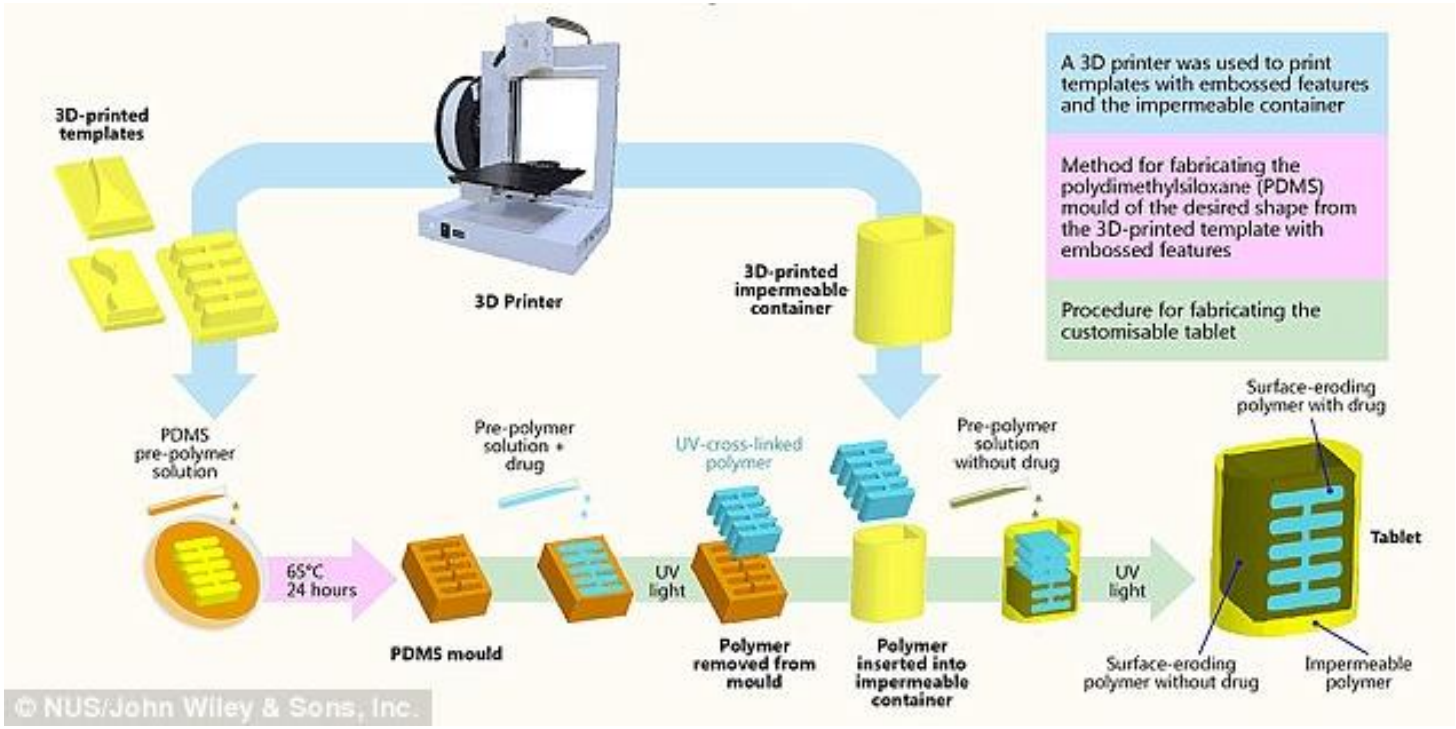


THE MARKET

- The world demand for 3D printers, materials and software is projected to increase by 21% percent per year and increased to $5 billion in 2017.
- Past → compared to other sectors, 3D printing technology played a minor role in healthcare. Healthcare only accounted for 1.6 percent of all investments made into the $700 million 3D printing industry. But - expected to grow to 21% over the next 10 years.
- Future → latest research shows more drastic development for health and medicine. Using 3D printing for medical applications could amount to a market value of $2.13 billion by 2020 (MarketsandMarkets.com).

3D PRINTING BASICS

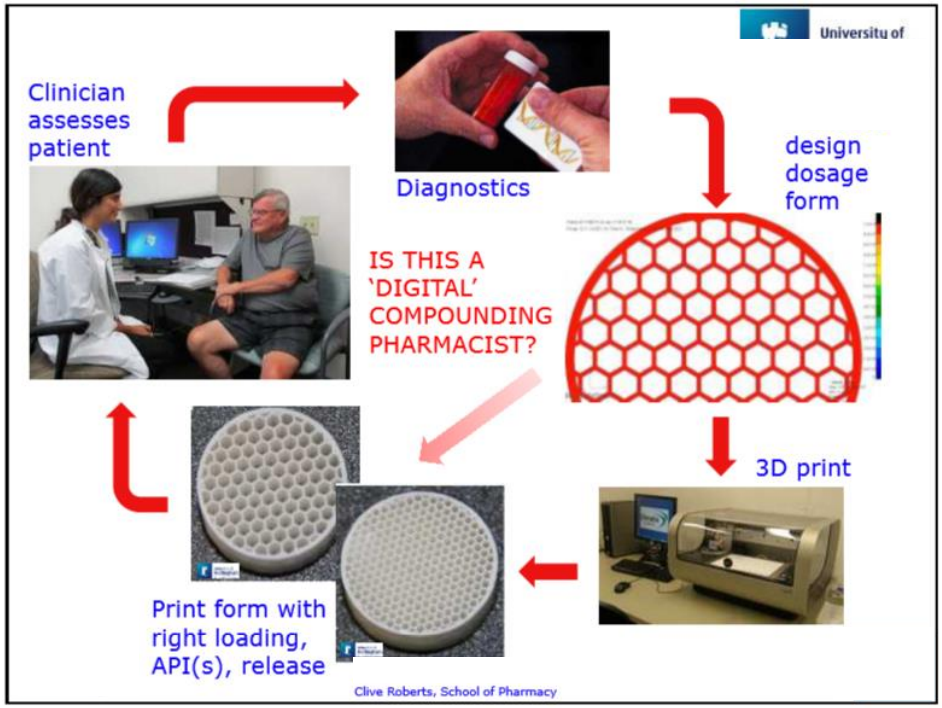
- 3D printing is a process that creates a three-dimensional object by building successive layers of raw material.
- Each new layer is attached to the previous one until the object is complete.
- Objects are produced from a digital 3D file, such as a computer-aided design (CAD) drawing or a Magnetic Resonance Image (MRI).
- The flexibility of 3D printing allows researchers to implement changes easily without the need to set up additional equipment or tools.



Example procedure for creating customisable tablets

PERSONALIZED DRUG DOSING

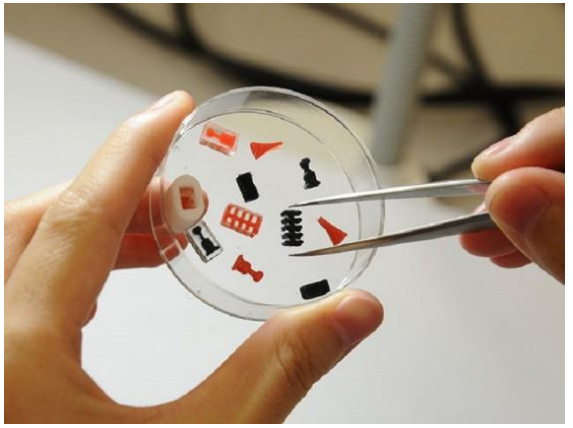
- Personalized 3D printed oral tablets – especially where patients respond to the same drugs in different ways.
- Use each patient's individual information e.g. mass, metabolism, age, race and gender - to produce their optimal medication dose and drug release rate.
- 3D printing enables printing of tablets as complex construct of layers/combination of polymers printed in different shapes - combination of drugs to treat multiple ailments simultaneously e.g. a multidrug polypill for cardiac ailments - enhances patient compliance.
- Personalized implants allows for printing implants that match patients' anatomical features - this technique is gaining traction for medical devices e.g. bone grafts.



UNIQUE DOSAGE FORMS & DRUG RELEASE PROFILES

- Challenge conventional tablet fabrication e.g. direct compression.
- Limitations of conventional approaches = low dosage, non-continuous release of drugs in the body, drugs released in large bursts or poor durability of tablets.
- Recently dosage form design fuelled largely by polymer science resulting in extended- and delayed-release tablets, transdermal systems, and long-acting implants.
- 3D printing introduces a novel element into dosage form development (i.e. digital control over the arrangement of matter) – create unique dosage forms. Using inkjet-based 3D printing technology to create limitless increasingly innovative dosage forms - striking changes in immediate-release, modified-release, and combination-drug products.
- Facilitate targeted, controlled and customised drug release: e.g. printing different polymeric stimulus-responsive or barrier layers or using computer software to generate a template that achieves desired release in specific patient.

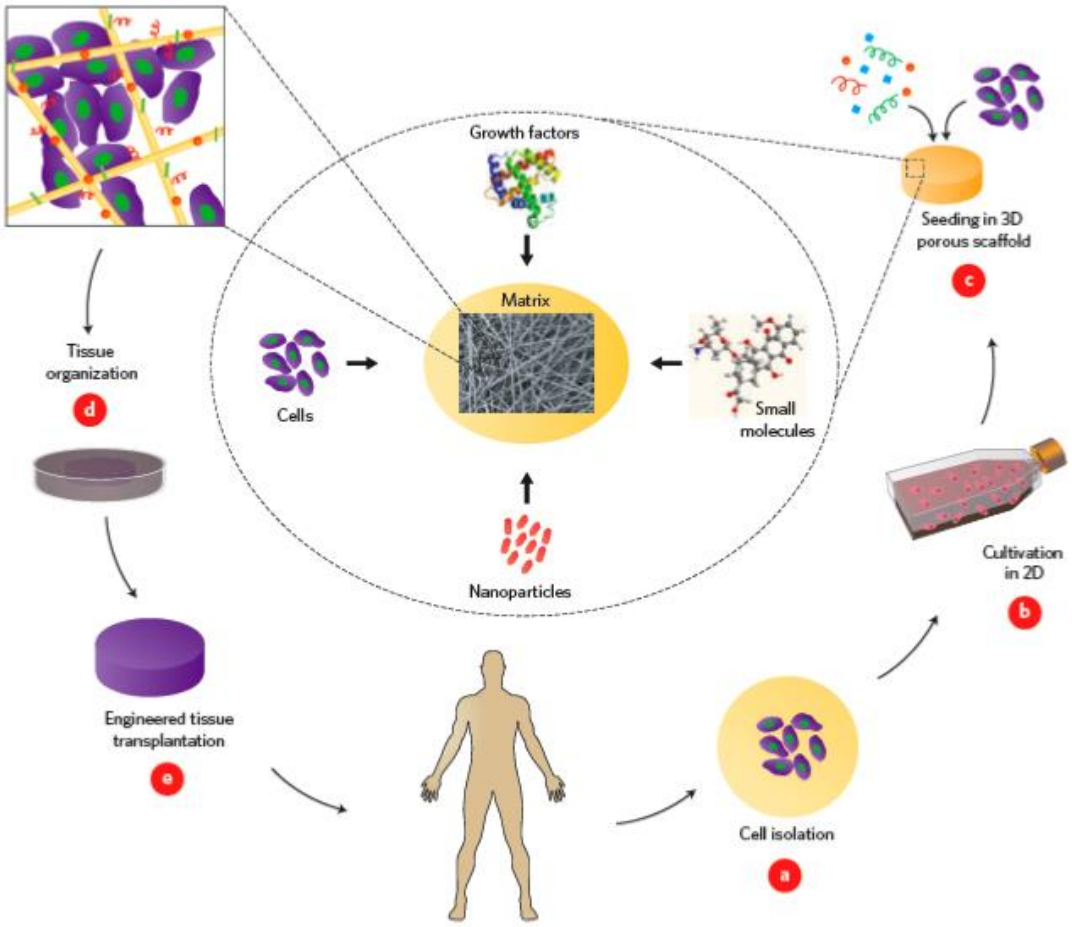
The first FDA approved 3D printed tablet for epilepsy, Spritam, now available in the US. Uses Aprecia's ZipDose Technology - formulation science combined with 3D printing to create a high-dose medication in a rapidly disintegrating form



A breakthrough fabrication method developed by scientists in Singapore can be adjusted to suit every patients' personalized medication. This invention is in the form of 3D printed pills (National University of Singapore)

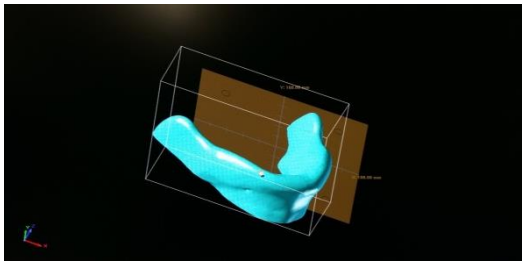
3D BIOPLOTTING FOR BIOINSPIRED TISSUE ENGINEERING

- 3D Bioplotting is a specialised arm of 3D printing that employs biological materials in computer-aided tissue engineering to create structures for enhanced biomimicry.
- Goal = achieve an effective combination of cell and bioactive components for restoration and/or improvement of biological functionality.



3D-BIOPLOTTING AT THE WADDP

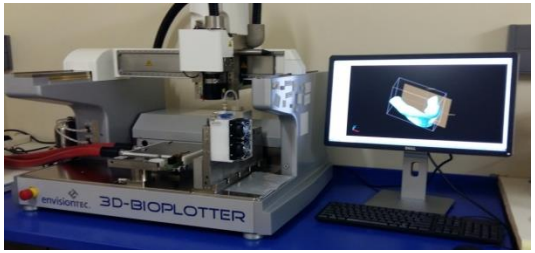
Design (CAD)



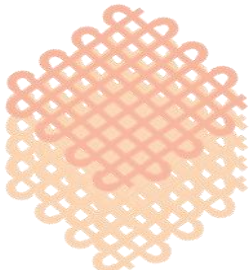
‘Bioink’

Matrix	+	Biological components
• Biomaterials • *Nanostructures* • Synthetic or natural		• Cells • Growth factors • Drugs

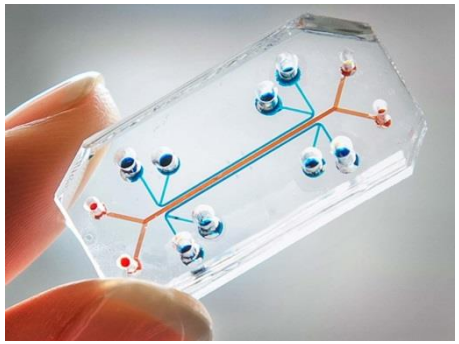
Bioprinting



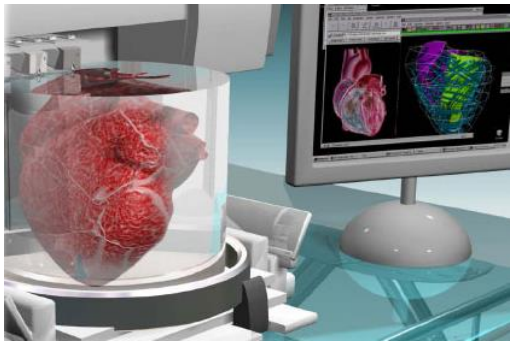
Layer-by-layer



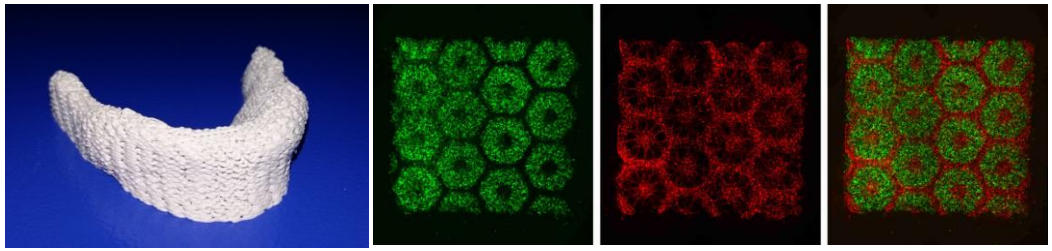
Applications



Organ-on-a-chip



Organ Printing



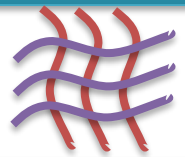
Tissue engineering

3D-BIOPLOTTING AT THE WADDP

Tissue Engineering of Biomaterials

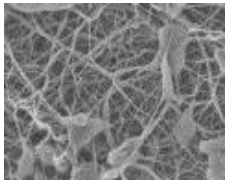
Natural/ Synthetic Polymers

Ceramics

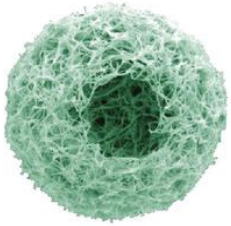


Hybrid/Composite

Fabrication Approaches for Nanostructured Material Design



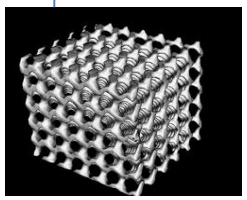
Electrospinning



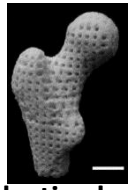
Thermally-induced phase separation

Sol-gel approach

Rapid Prototyping Techniques



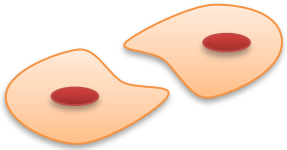
Stereolithography



Selective laser sintering

3D Printing

Biorelevant Nanosized Additives



Cells

Bioactive Nanoparticles (e.g. CaP, HA, MNPs, nanodiamonds), growth factors

Implantation/injection at Tissue Defect Site

3D-PRINTED CONTROLLED RELEASE TRITHERAPEUTIC TABLET

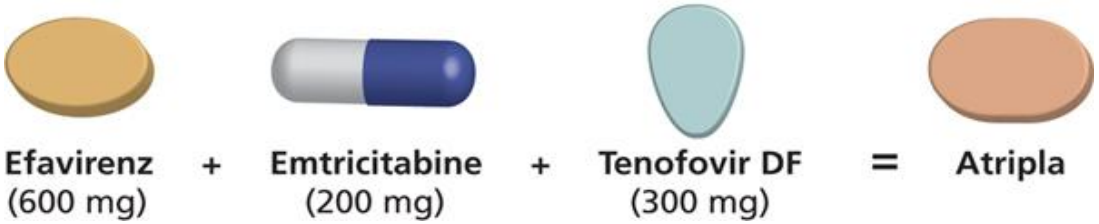
- A fixed three drug dose combination of the anti-HIV drugs is required that provides controlled levels of drugs within the therapeutic window with reduced side effects.

Solution:

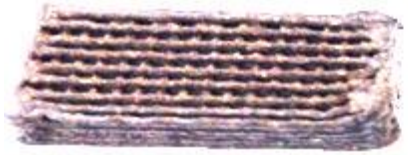
- Designed a novel 3D-printed (3DP) Fixed-Dose Combination tablet

Key Features:

- Humic acid-polyquaternium 10 complex (HA-PQ10) as the bio-ink to achieve controlled release of three drugs: efavirenz (EFV), tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) as the model drug regimen.



Conventional anti-HIV FDC

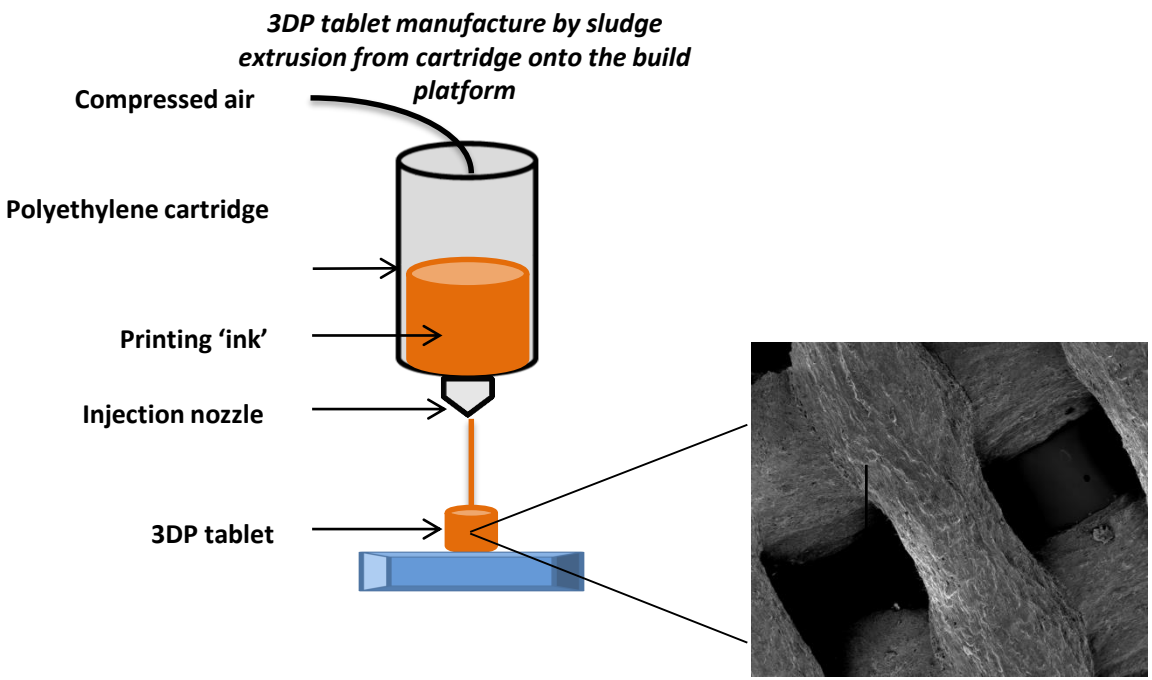


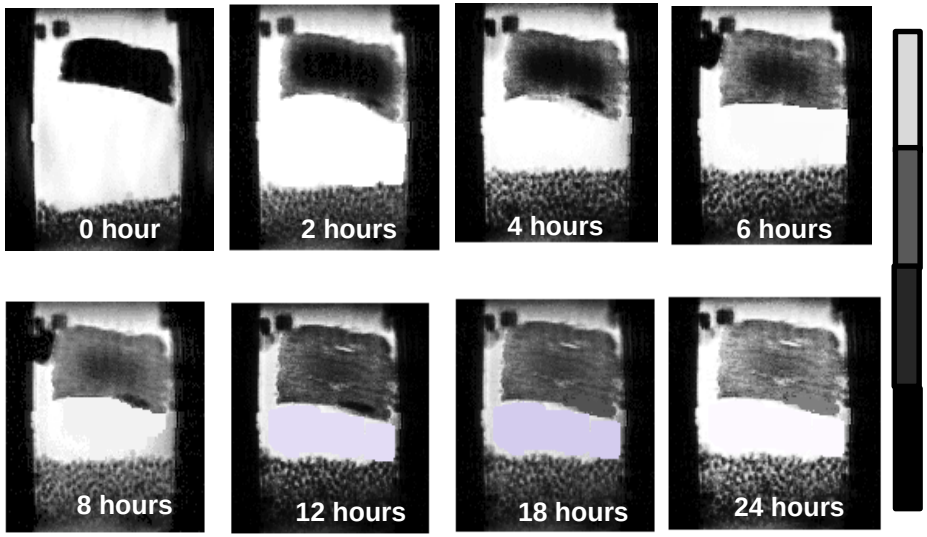
EFV, TDF and FTC mixed in one sludge (preferred configuration)

Separate layers of EFV, TDF and FTC

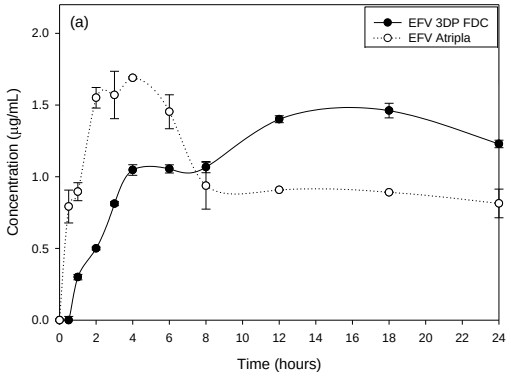
Example of 3DP anti-HIV FDCs

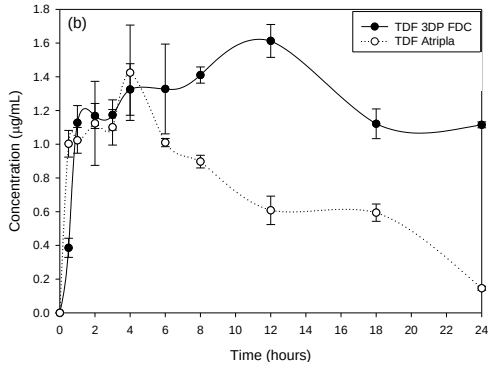
Customized Manufacture and Drug Release from the 3D Printed FDC

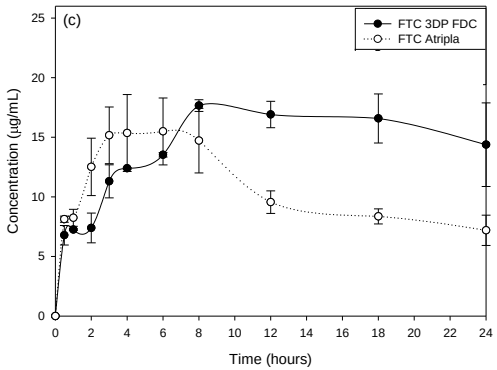




Controlled hydration of the 3D-Printed tablet architecture







In vivo drug levels highlighting enhanced relative bioavailability of all 3 anti-HIV drugs from the 3DP FDC vs. Atripla®

A 3D BIOPLOTTED PVA-PAA HYDROGEL LOADED-POLYCAPROLACTONE SCAFFOLD FOR THE DELIVERY OF HYDROPHILIC *IN-SITU* FORMED SODIUM INDOMETHACIN

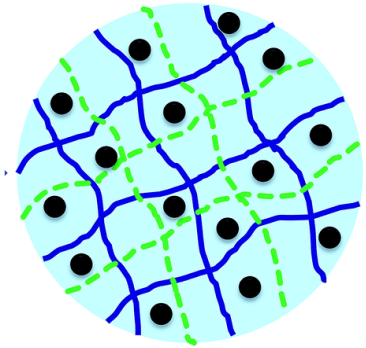
- The addition of anti-inflammatory agents to 3D bioplotted structural support scaffolds → acute treatment of site-specific inflammation + long-term structural support to allow for adequate tissue repair and rehabilitation.

Solution:

- NSAID incorporated into the architecture of the PCL scaffold via a nanostructured semi-IPN composed of PVA and PAA.

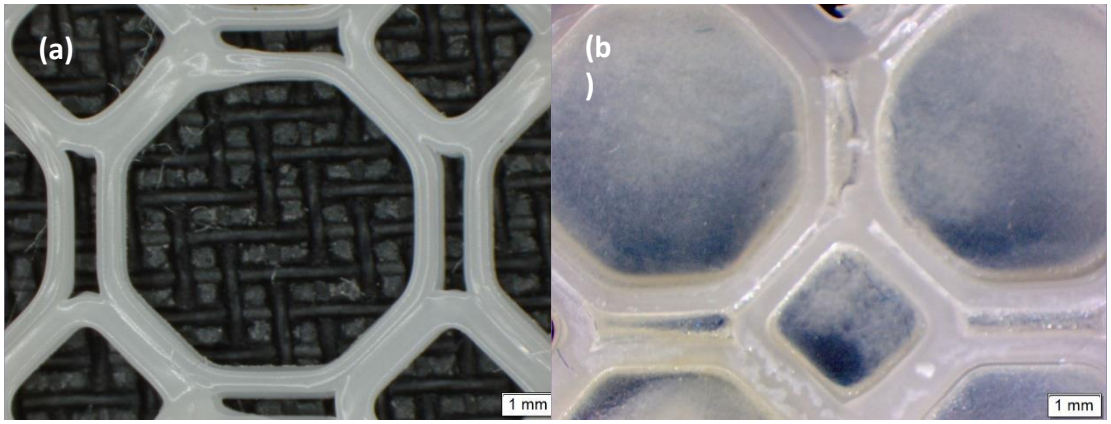
Key Features:

- Nano-enhancement: Nanostructured semi-IPNs allow for the production of a relatively dense hydrogel matrix + strong mechanical properties + more efficient drug loading.
- The prepared PCL-PVA-PAA (PPP) scaffold is proposed as a structural support system for load-bearing tissue damage where inflammation is prevalent.

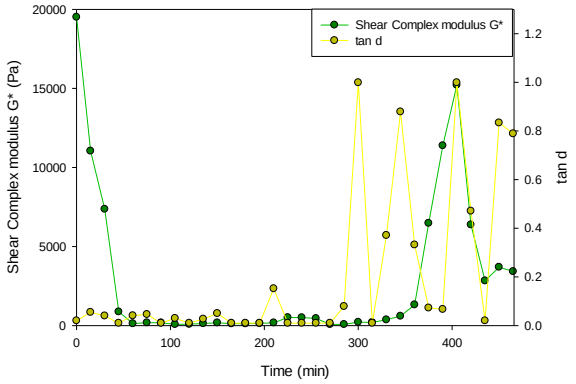


Nanostructured IPN with sodium indomethacin for incorporation in PCL scaffold

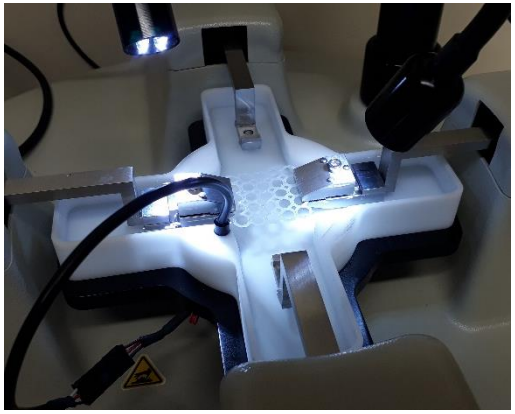
Physicomechanical and Drug Release Attributes of PPP Scaffold

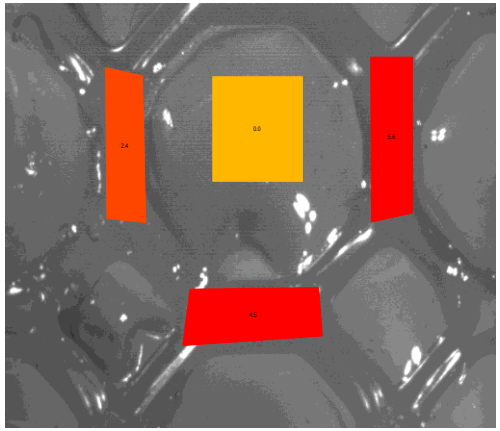


Microscopic images of (a) the 3D-printed PCL scaffold and (b) the PPP scaffold highlighting uniform adhesion of the PVA-PAA hydrogel within the octagonal structure

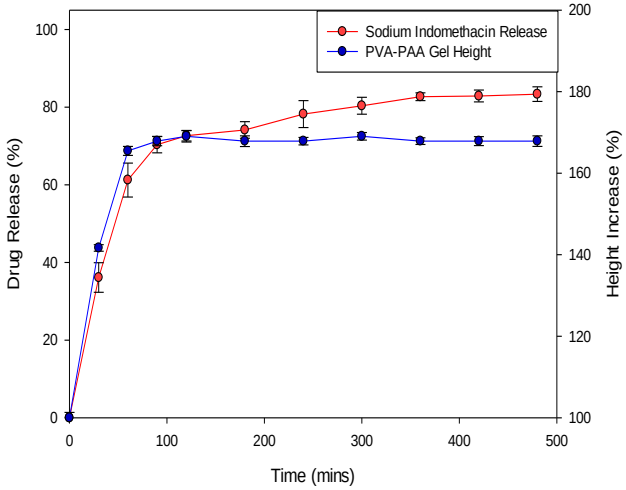


Tan δ & the shear complex modulus (G) approached 1.00 - the hydrogel was fully hydrated with appreciable resistance to deformation*





Uniaxial strain testing and tracking of the hydrated PPP scaffold depicting biomimetic physicomechanical attributes



Inflammation-sensitive scaffold hydration and release of anti-inflammatory agent

A BIOINSPIRED 3D-BIOPLOTTED DEVICE FOR BURN WOUNDS

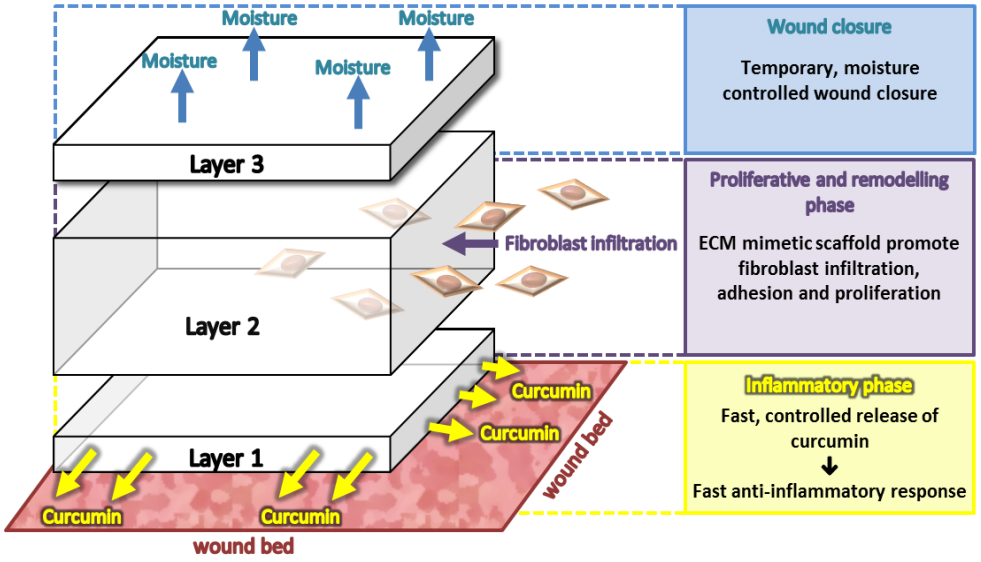
- According to the WHO *ca* 11 million worldwide cases of burns requiring medical (Burns Report, WHO, 2014).

Solution

- 3D bioplotted wound healing device with superior wound regenerating properties incorporating cell seeding and growth factors.

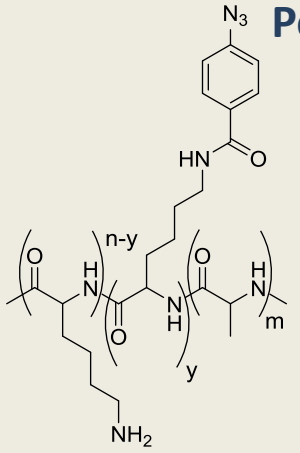
Key Features:

- Combines 3 different layers into one device.
- Provide controlled release of active compounds; enhanced biointegration of regenerated tissue.
- Employs varied nano- to micro-architectures, biomimetic polymers, active compounds and biomolecules to provide an optimal wound healing environment.



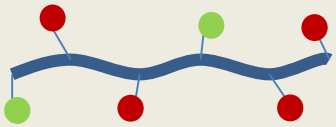
Conceptual design of final 3D Bioplotted Device for burn wounds

Design of the 3D Bioplotted Device for Burn Wounds



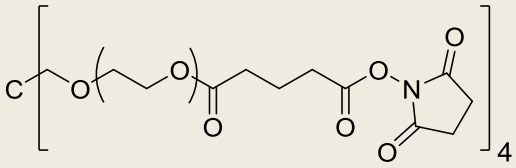
Poly(Lys)$_n$(Ala)$_m$

- Lys:Ala 60:40
- Biocompatible (Cell adhesive)
- Antimicrobial

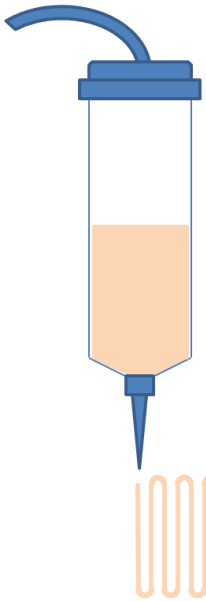


4-Arm PEG-SG

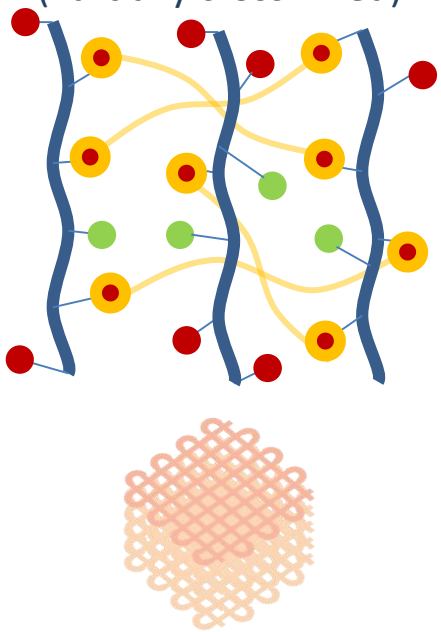
- Biocompatible (Bioinert)



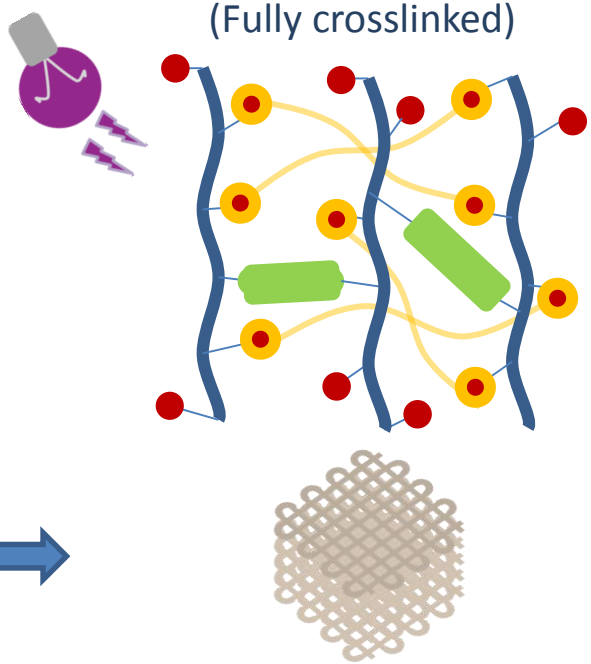
Precursor Hydrogel
(Partially crosslinked)



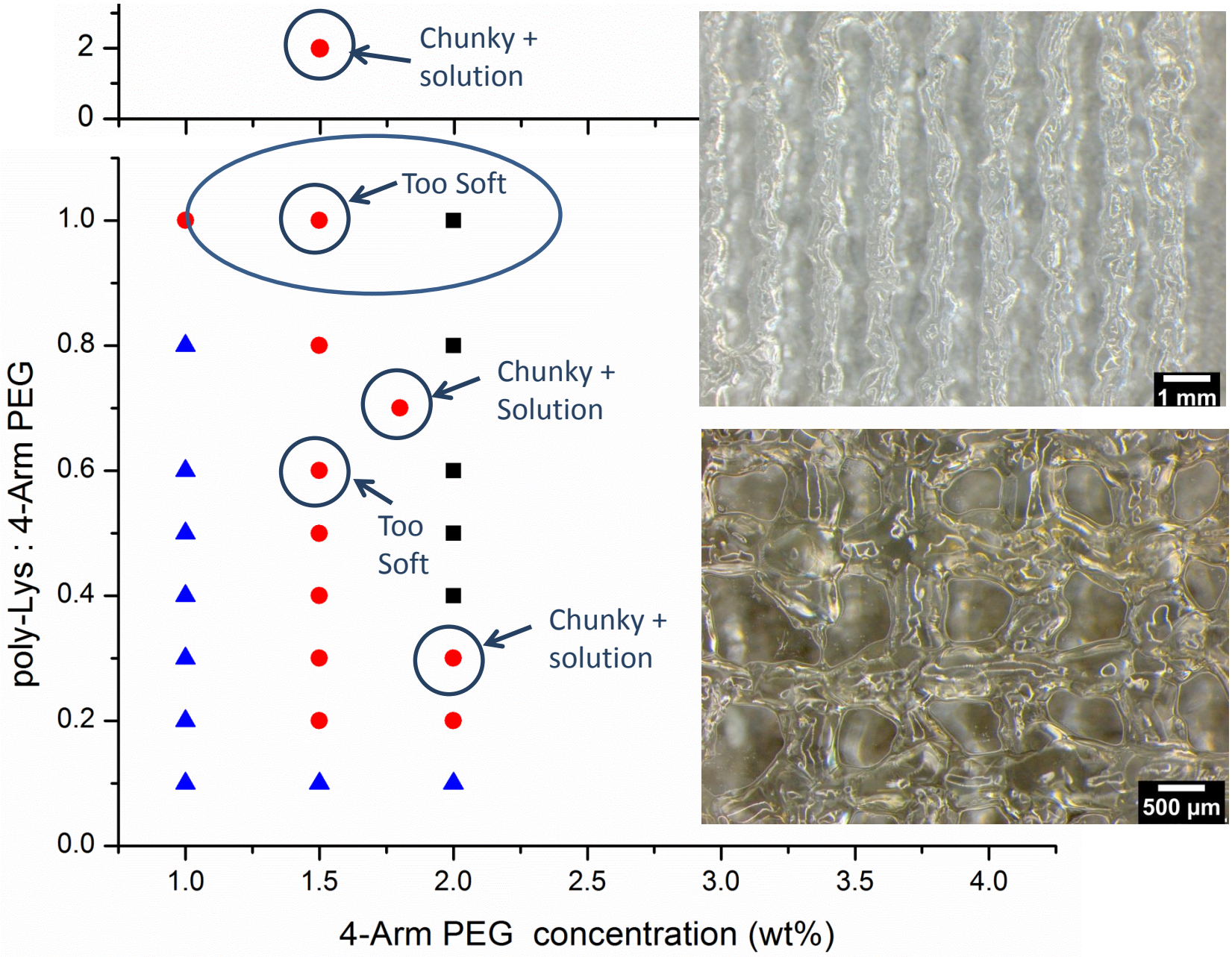
3D Structure
(Partially crosslinked)



3D Scaffold
(Fully crosslinked)



Hydrogel Formation



A 3D BIOPLOTTED IMPLANTABLE DRUG DELIVERY SCAFFOLD FOR APPLICATION IN BONE TISSUE ENGINEERING

- Traditional treatments for orthopedic defects due = orthopedic implants, allografts, and autografts → challenges of infection, inadequate healing following invasive surgeries, inadequate donors, and morbidity at the donor site.

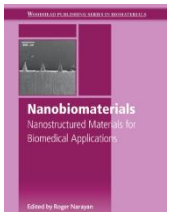
Solution:

- Design a 3D bioplotted pseudo-bone drug delivery system – inserted at the site of bone fracture (model = clavicle).

Key Features:

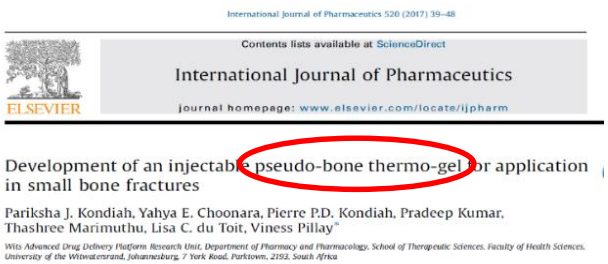
- The implantable system comprised of a copolymeric biomatrix based on a thermoresponsive gel (thermogel) - loaded with a statin drug.
- Nano-enhancement: Nano-functionalized with growth factors and cells.

Application of the 3D Bioplotter for fabrication of a jaw bone scaffold

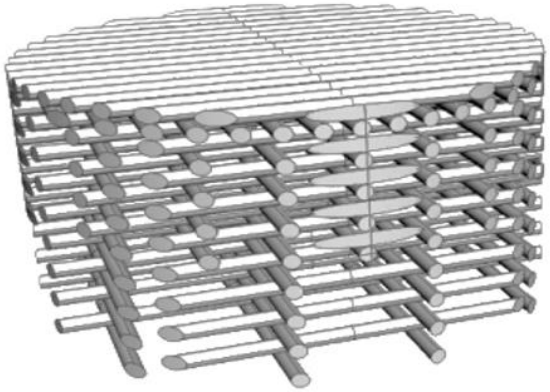


Use of nanostructured materials in hard tissue engineering

L.C. du Toit, P. Kumar, Y.E. Choonara, V. Pillay
University of the Witwatersrand, Johannesburg, South Africa

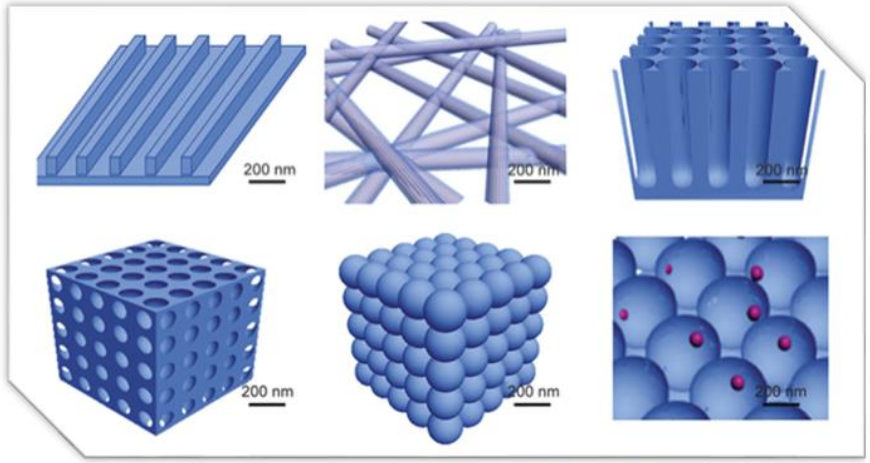


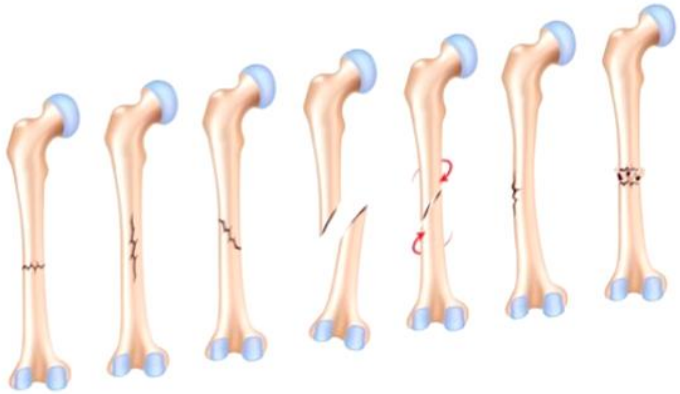
Mechanistic Overview of the 3D Bioplotted Scaffold in Bone Fractures



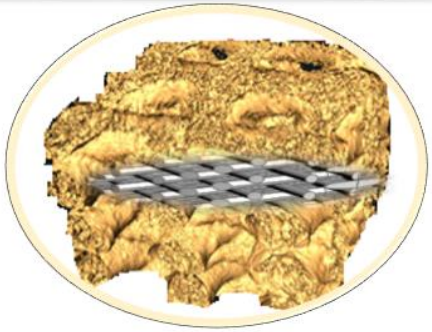
Schematic model representing the 3D printed implantable scaffold for bone tissue engineering and drug delivery

Morphological structural size arrangements of 3D printed scaffold: Macrostructure, microstructure and nanostructure arrangements





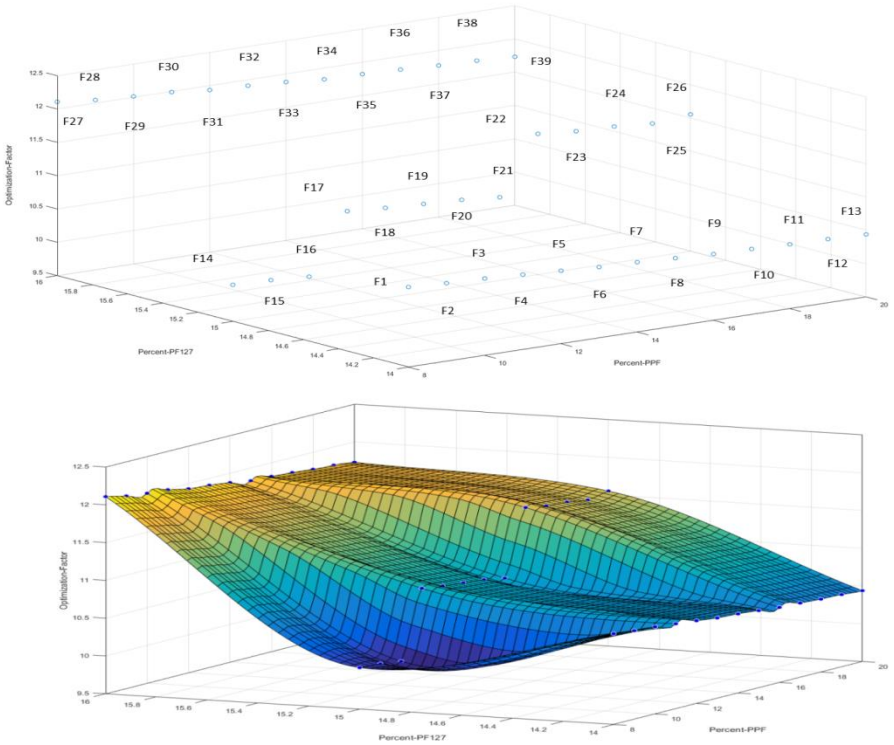
Types of fractures depicted above for application of 3D printed implantable scaffold: (Transverse Linear) (Oblique –nondisplaced) (Oblique displaced) (Spiral) (Greenstick) (Comminuted)



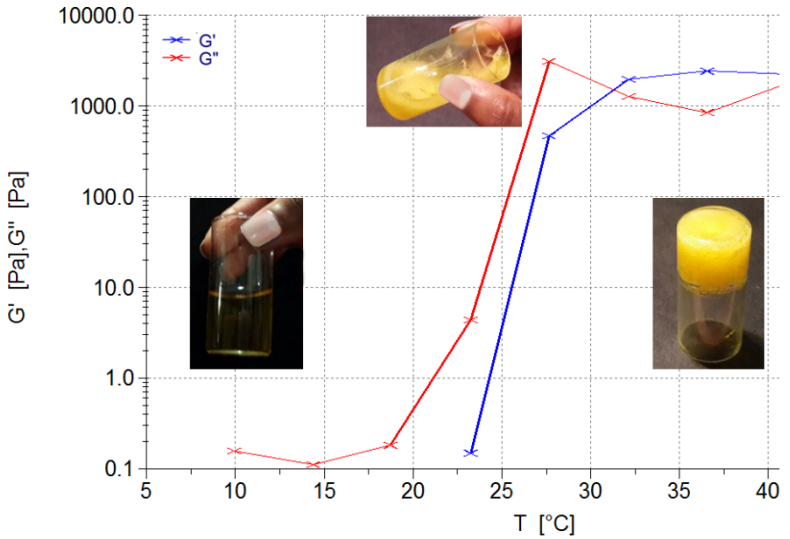
Implanted 3D scaffold at the site of fracture

Optimization and Analysis

- 39 thermo-gel designs were loaded with a statin drug and optimized for 3D bioprinting – using MATLAB®.
- Variables of PPF and PF127 were studied in response to duration of release of simvastatin and thermo-gelation temperature.



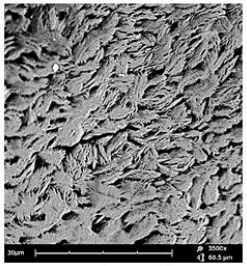
3D representation of the designed formulations using a cubic function surface plot: highest point = optimum polymer concentrations

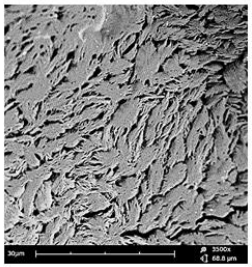


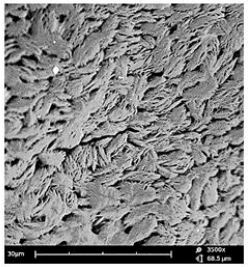
Rheological evaluation of the 3D bioink in relation to change in temperature = thermogelling

Ex Vivo Evaluation in a Bone Model

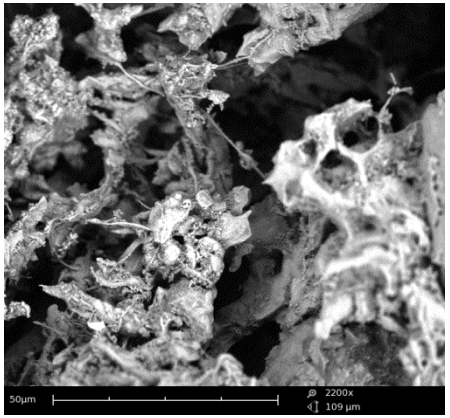
- *Ex vivo* evaluation of the 3D bioprinted pseudo-bone scaffold via ultrasound and X-ray
- Evaluation on a fracture-induced human clavicle bone → cell proliferation, fracture filling and bone repair

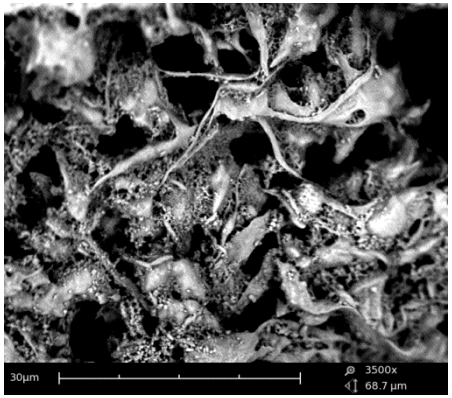

Layer 1


Layer 2


Layer 3

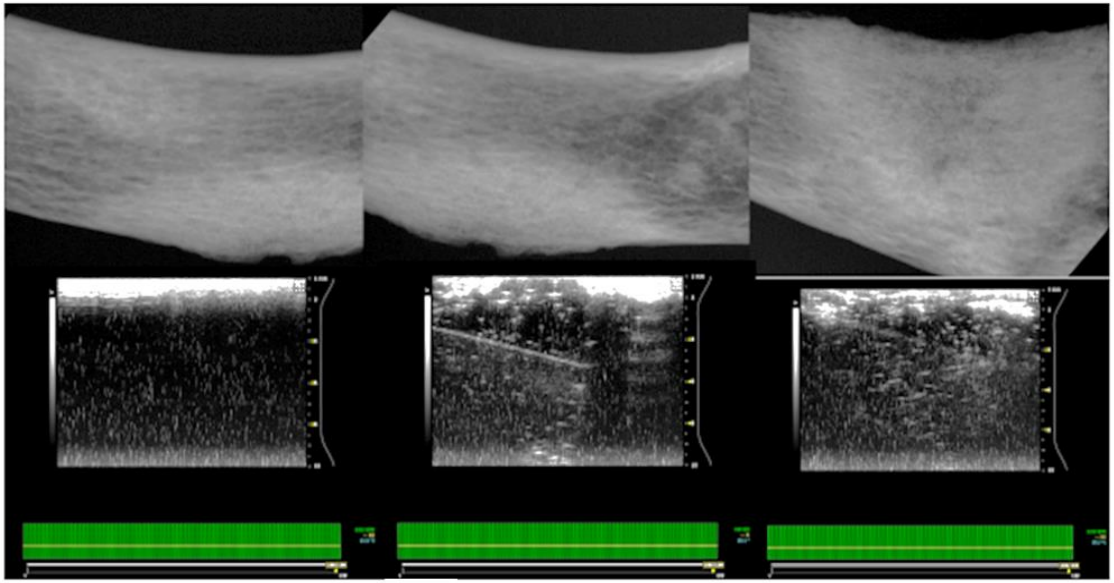
SEM analysis of scaffold layers → nano-architecture and inner porous nature = nutrient diffusion + cell attachment and proliferation





SEM visualization of osteoblast-like cell attachment to the bioplotted scaffold

Before fracture ***After fracture*** ***After 3D scaffold treatment***



X-ray and ultrasound images on a human clavicle osteoporotic female bone showing bone regeneration

IN SITU CONJUGATION-CO-FABRICATION OF BIOARCHETYPES EMPLOYING 3D BIOPLOTTING

- Demand for progressive single-step fabrication approaches for production of nanostructured bone scaffolds.

Solution:

- Engineer a single-step *in situ* conjugated nanostructured polymeric scaffold employing 3D bioplotting with inclusion of nano-additions.

Key Features:

- A polymeric scaffold engineered *in situ* employing sodium alginate interacted with a poly(ethyleneimine) on bioprinting to form a polyelectrolyte complex as a bio-ink.
- Nano-enhancement: Nanostructuring via silica (Si), nanoclay (NC) or hydroxyapatite (HA) in the bio-ink as temporal inorganic support component + ultimate enhancement of osteoinduction.

Society For Biomaterials

A 3D bioprinted *in situ* conjugated-*co*-fabricated scaffold for potential bone tissue engineering applications

Mduduzi N. Sithole, Pradeep Kumar, Lisa C. du Toit, Thashree Marimuthu, Yahya E. Choonara, Viness Pillay

Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown 2193, South Africa

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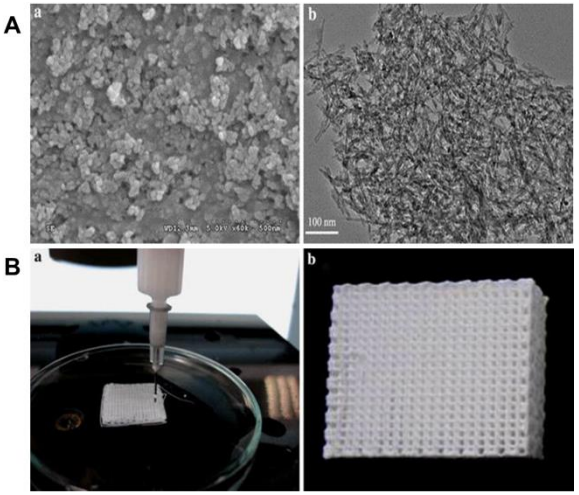
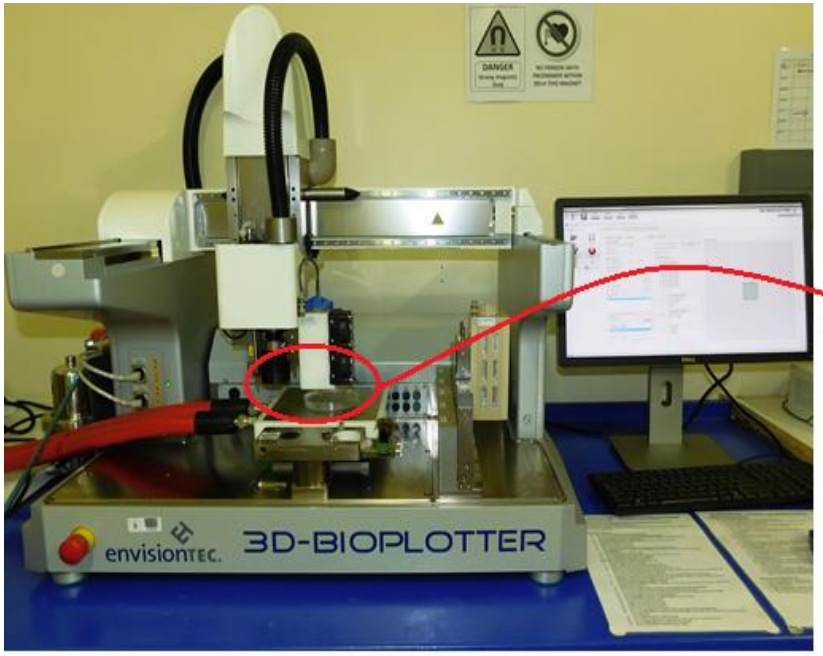
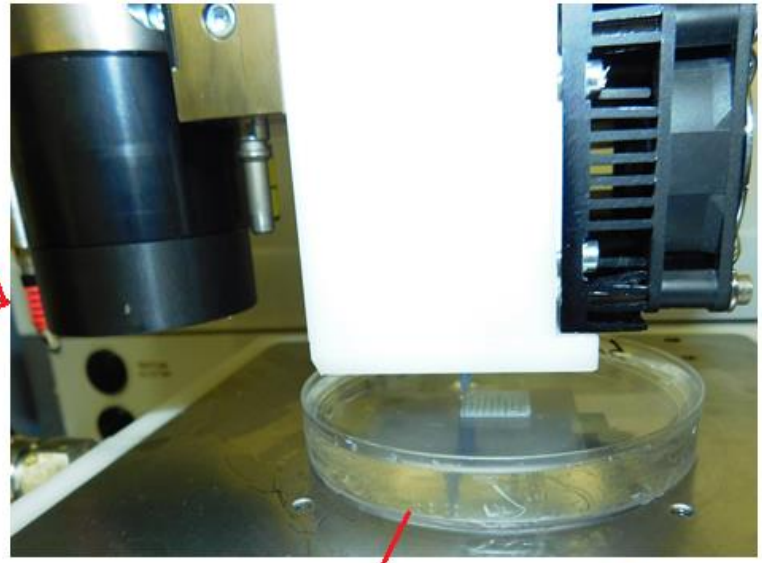


(a) SEM and (b) TEM of Nano-HA

(a) 3D Printing to produce (b) nanocomposite scaffold

In Situ Conjugation-Co-Fabrication of the Nanostructured Bioarchetypes





Anionic NaAlg/Si solution

Cationic PEI solution

3D print cartridge containing printable solution

'Ladder-like'

Polyelectrolyte complex

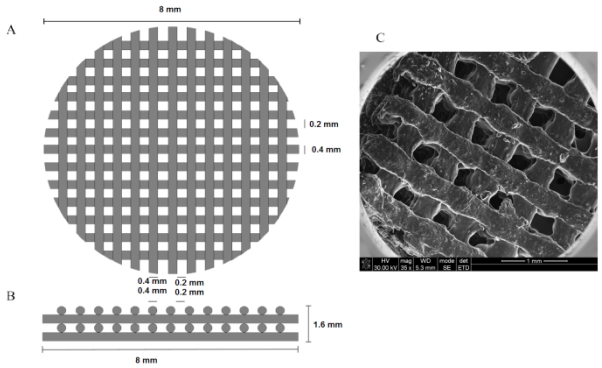
Scaffold

Polyanion

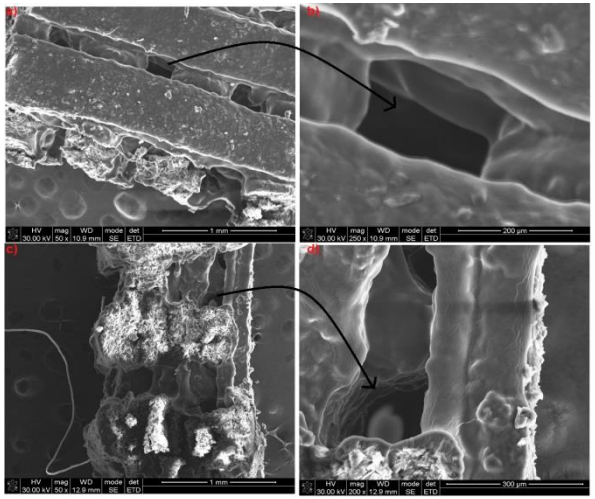
Polycation

Nanostructures (Si/NC/HA)

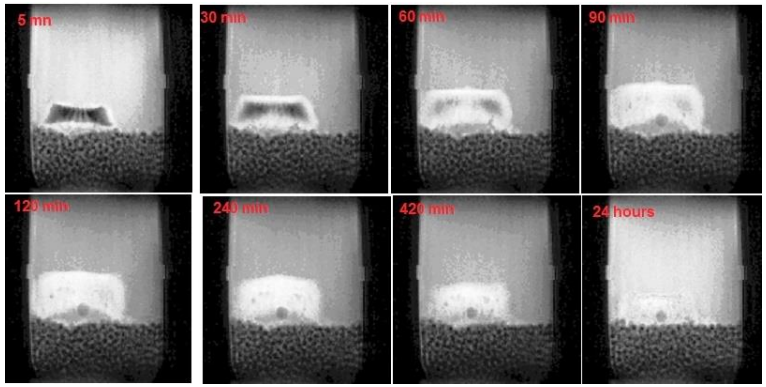
Architectural Visualization and Physicomechanical Analysis



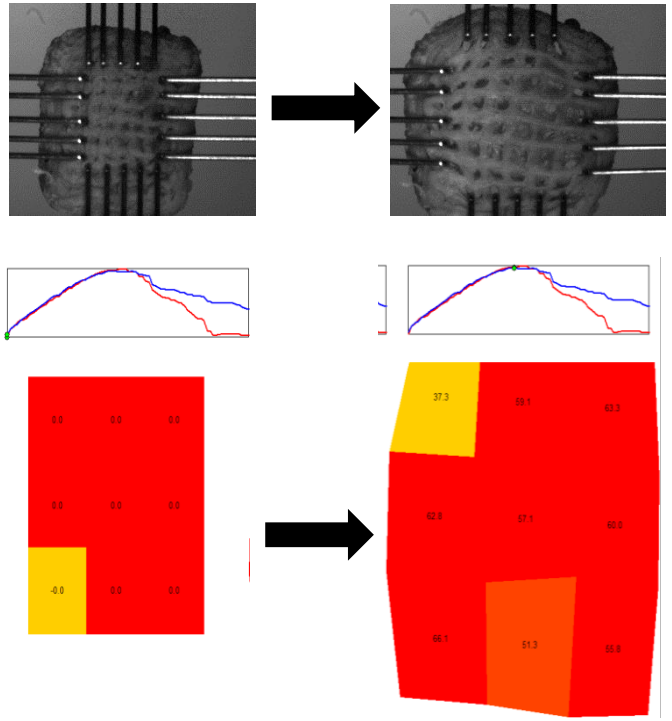
A. Surface and B. Cross-sectional schematic of the proposed design of the bioplotted scaffold. C. SEM image of the surface section of the bioplotted Alg-PEI/Si or NC or HA scaffold



Scaffold roughness modulates the biological response through enhancement of cellular adhesion

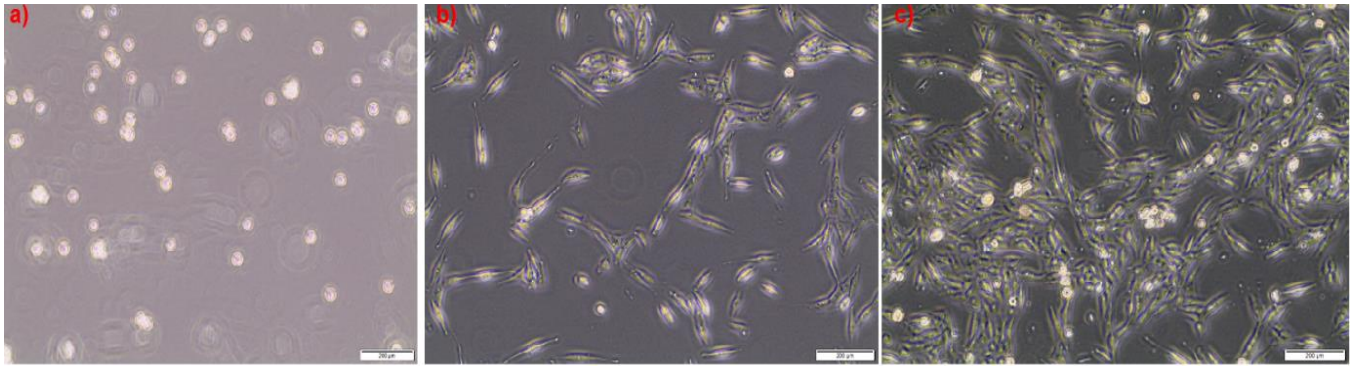


Magnetic Resonance Imaging of the bioplotted scaffold

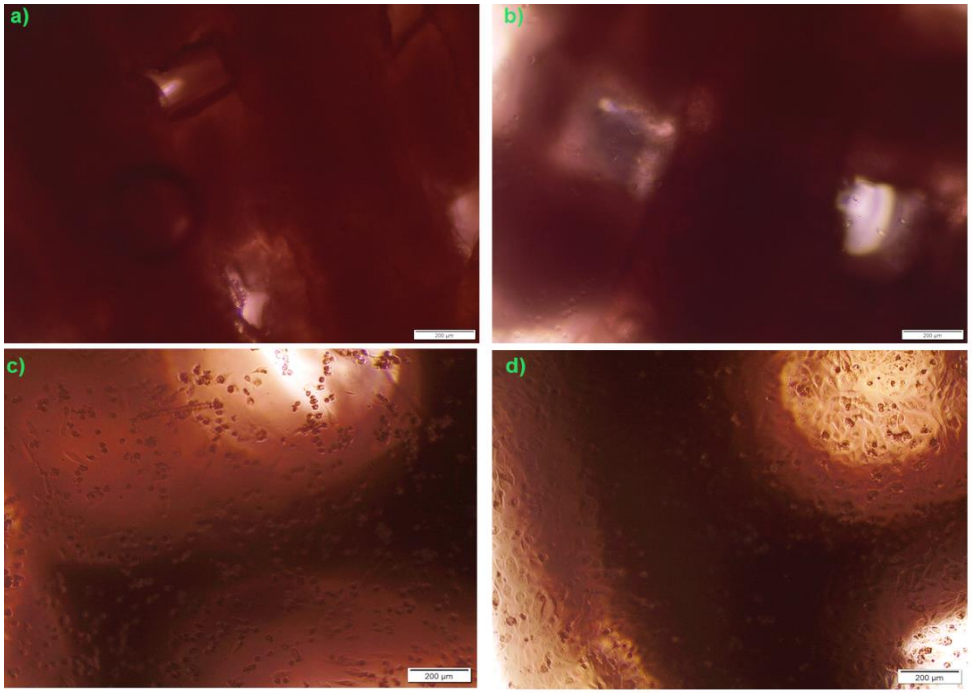


BioTester images and corresponding real-time image analysis and force-displacement graphs representing scaffold strength a) before application of force and b) during maximum stretch

Analysis of cell differentiation; and adherence and growth to the in situ conjugated-co-fabricated nanostructured polymeric scaffold



Assessment of osteoblast-like MG63 cell differentiation: (a) Day 0 (b) Day 2 (c) Day 3 - The osteoblast-like MG63 cells were successfully seeded in the bioplotted scaffolds at Day 3



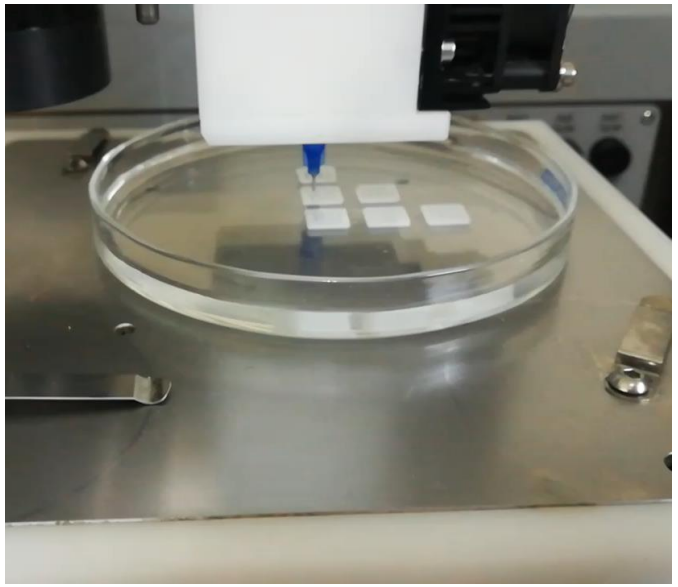
(a) Scaffold without cells. (b-d) Day 1-7 showing increasing confluence of osteoblast-like MG63 cells on the scaffold. After Day 1 cells demonstrated adherence and growth to the printed scaffold. From Day 3 - cells formed colonies which increased density (Day 7)

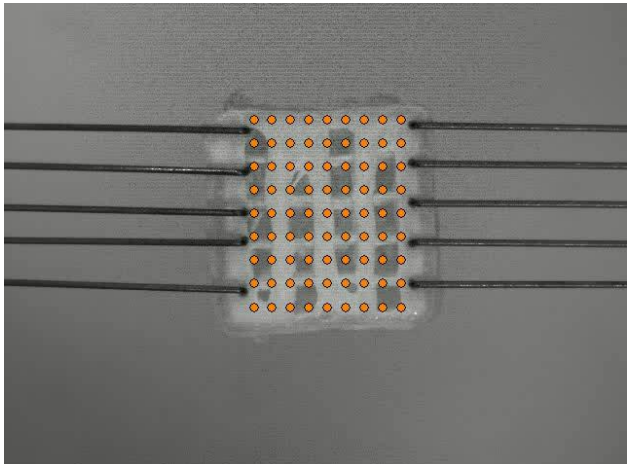
APPLICATION OF CHEMICAL MODIFICATION FOR ENHANCED PHYSICOMECHANICAL PROPERTIES OF 3D BIOPLOTTED SCAFFOLDS

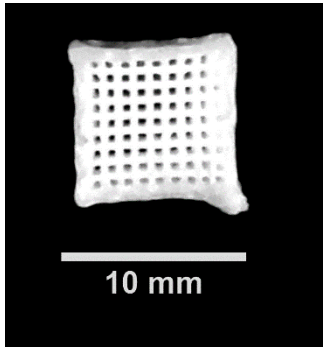
- Progressive approaches required for fine-tuning physicomechanical properties of bioplotted scaffolds.

Solution:

- Surface chemical modifications and subsequent functionalization of 3D printed scaffolds (e.g. aminolysis/hydrolysis with chitosan attachment; alkynylated and grafted with zwitterionic PCBMA-N_3).
- Identified a plasticizer - improved the printability of the bioink and mechanical properties of the printed construct.
- Nano-enhancement: Incorporation of nano-strengthening agents for enhanced physicomechanics.







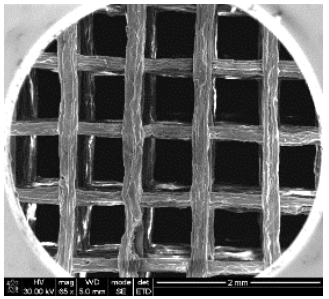
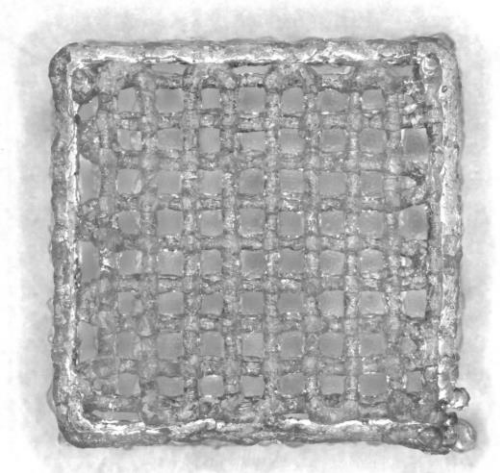


Image and SEM of 3D bioplotted scaffold

3D PRINTED, ARTIFICIAL EXTRACELLULAR MATRIX FOR POTENTIAL NEUROREGENERATION

- Nerve regeneration capabilities of the human nervous system are insufficient
  - Incomplete repair and regain of function
  - No successful therapeutic scaffold for regeneration of damaged nerves

Solution: develop a 3D printed, self-assembled biomaterial artificial extracellular matrix (aECM) for nerve regeneration



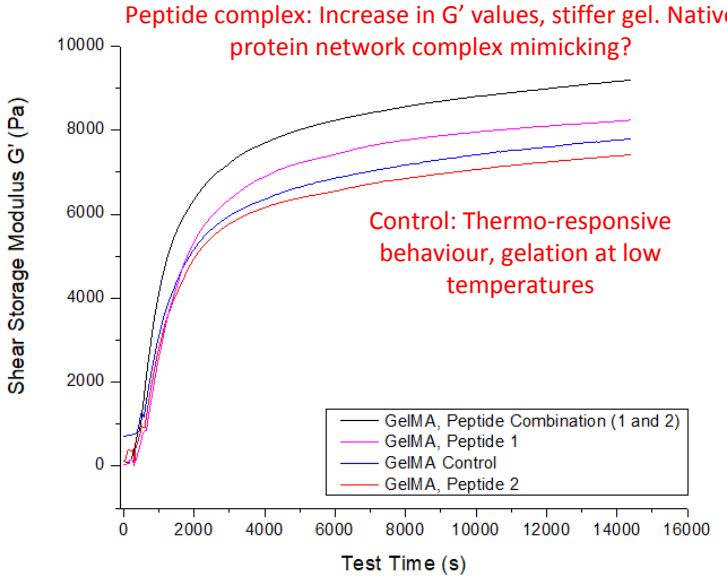
3D bioplotted peptide hydrogel

Key Features:

1) <u>Polymer (GelMA)</u>
- Semi-synthetic hydrogel: Provides RDG groups for cellular adhesion
- Thermo-responsive physical crosslinking behaviour
- UV crosslinkable hydrogel

2) <u>Peptide-Hydrogel</u>
- Successful incorporation of native peptides
- Maintenance of original polymer behaviour
- 3D printable system

Gelation of Peptide-Hydrogel combinations at a 10°C temperature hold over 4 hours (n=3)



CONCLUSION AND FUTURE DIRECTION

- The largest challenge in this field - ability to balance all performance objectives for successful scaffold integration within areas of biological and physical complexity.
- A large volume of current research has shown marked advancement in one direction, e.g. biocompatibility BUT with inadequate results in mechanical strength.
- Integration of appropriate porosity into scaffolds to improve cell viability and promote healthy vasculature throughout the repaired tissue.
- Materials that can closely mimic the properties of the natural tissue are essential (biocompatibility + mechanical loading requirements).
- Further future research into novel nanostructured bio-inks.
- Further advancement to 3D-bioprinting of organs - experts project that science is less than 20 years away from a fully functioning 3D printed heart. Currently - 3D printed heart is still challenged by the intricate nature of vasculature.