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Polyhydroxyalkanoates for Osteochondral Tissue Engineering: A Biomimetic Approach to Osteoarthritis Treatment

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Introduction



- Global plastic production **>300 million tonnes** annually, **increasing ~5 %** each year[1].
- Over 85 % of petroleum-based plastics remain in the environment posing **significant ecological burdens**[2,3].
- Bioplastics represent only ~1 % of plastic production according to the 2020 Global Bioplastic Market Report [4].

Polyhydroxyalkanoates (PHAs):

- Microbially produced, biodegradable and bioresorbable polyesters
- A **sustainable alternative** to fossil-based resources
- **Highly tuneable** structural, mechanical, and thermal properties

Fantastic potential for **biomedical applications** – optimisation of biosynthesis and blending with other PHAs can tailor the polymers to specific applications.

The Clinical Challenge:

There is an extreme clinical need for **effective osteochondral repair** strategies to treat **early-stage osteoarthritis**.

- Traditional treatments often fail to restore native articular cartilage (AC) function
- Gold standard = awaiting final "joint death" necessitating Total Joint Replacement
- Implants have limited lifespans and younger patients are frequently requiring more complex revision surgeries

This research aims to develop **biomimetic PHA osteochondral scaffolds** replicating the native gradient osteochondral properties to facilitate tissue regeneration.

Scaffold Fabrication and Evaluation:

Articular Cartilage (AC) Phase:

Fabrication: Electrospinning of P(3HB)/MCL-PHA blends has been investigated to achieve biomimicry of the native AC extracellular matrix structure (fig. 4).

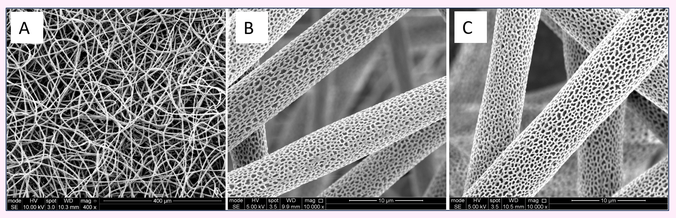


Figure 4 – (A) SEM micrographs of randomly oriented electrospun P(3HB)/MCL-PHA blends. Scale bar = 400 µm. (B and C) Characteristic surface pitting. Scale bar = 10 µm.

SEM was used to evaluate **optimal polymer blends** and **electrospinning parameters** to achieve specific structural requirements for the various AC regions.
Distinct **surface microporosity (pitting)** observed was attributed to rapid chloroform volatility and could enhance cellular adhesion and facilitate nutrient exchange.

Polymer Production

Objective: Optimisation of PHA yield and properties through **controlled fermentation processes**

- Poly(3-hydroxybutyrate) (P(3HB)) and elastomeric medium chain length (MCL)-PHA were produced (fig.1)
- Temporal profiles (fig.2) were constructed to evaluate fermentation kinetics on polymer yield and properties

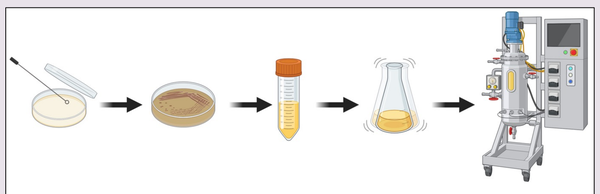


Figure 1 – Schematic of laboratory-scale PHA production. Single colonies of Wild-type bacteria were used to produce initial inoculum and were acclimatised with media and substrate prior to bioreactor inoculation. Fermentations were conducted in a 7L Applikon bioreactor, monitored and controlled by the Applikon Levit Flex bioprocess controller.

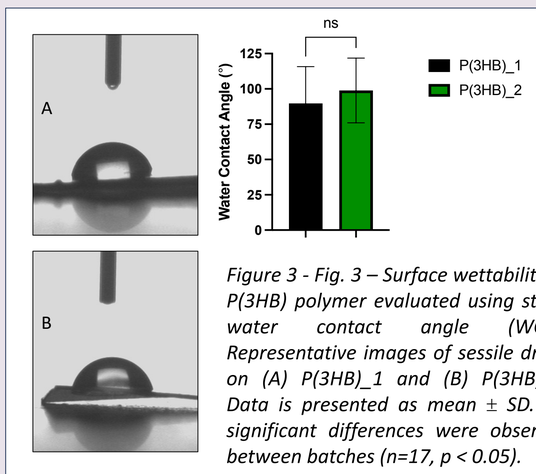


Figure 3 - Fig. 3 – Surface wettability of P(3HB) polymer evaluated using static water contact angle (WCA). Representative images of sessile drops on (A) P(3HB)_1 and (B) P(3HB)_2. Data is presented as mean ± SD. No significant differences were observed between batches (n=17, p < 0.05).

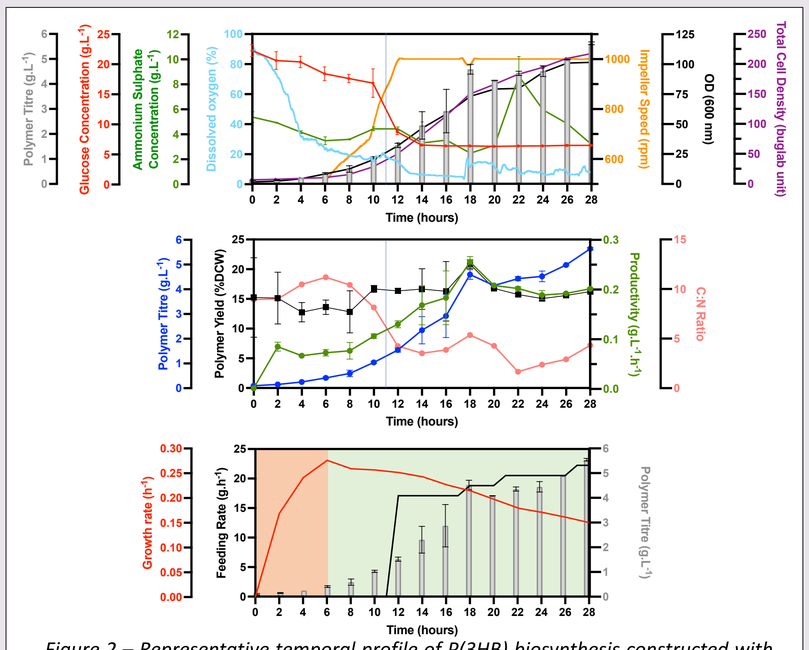


Figure 2 – Representative temporal profile of P(3HB) biosynthesis constructed with real-time data monitoring and temporal samples obtained in duplicate every two hours for determination of optical density (OD), carbon (C) and nitrogen (N) concentrations, polymer yield, and productivity. Carbon source was provided by controlled feeding depending on real-time dissolved oxygen (DO) data and insights from previous fermentations. Logarithmic DO data was used to calculate bacterial growth rates to delineate the distinct phases of bacterial growth (orange) and PHA production (green). This was compared to feeding rate and polymer titre (bottom).

Key Findings:

Characterisation confirmed production of high-purity PHAs with properties consistent with literature.

A trade-off was observed between biomass productivity and polymer quality during P(3HB) production

- Higher C:N ratios enhanced polymer accumulation and yield
- Lower C:N ratios enhanced crystallinity and thermal stability.

Table 1 – Physicochemical and mechanical characterisation methods and results of produced P(3HB).

	Technique	Results	
Chemical Characterisation	^{1}H Nuclear Magnetic Resonance	Confirmation of monomeric composition through successful identification of monomolecular organic compounds and evaluation of functional groups.	
	Attenuate Total Reflectance – Fourier Transform Infrared Spectroscopy		
Structural Evaluation	Gel Permeation Chromatography	Molecular Weight	100 – 1,000 kDa
		Polydispersity Index	1.5 - 2
Thermal Characterisation	Differential Scanning Calorimetry	Melting Temperature	~177 °C
		Glass Transition Temperature	~5 °C
		Crystallinity	55 – 80 %
	Thermogravimetric Analysis	Degradation Temperature	~250 °C
Mechanical Characterisation	Tensile Testing	Tensile Strength	14.2 – 15.8 MPa
		Elongation-to-break	1.45 – 1.49 %
		Young's Modulus	1.79 – 2.17 GPa

Scaffold Fabrication and Evaluation: Subchondral Bone (SCB) Phase

Fabrication: Melt extrusion 3D printed P(3HB) composites reinforced with graphene nanoplatelets (GNP) at 1, 3, and 5 wt%.

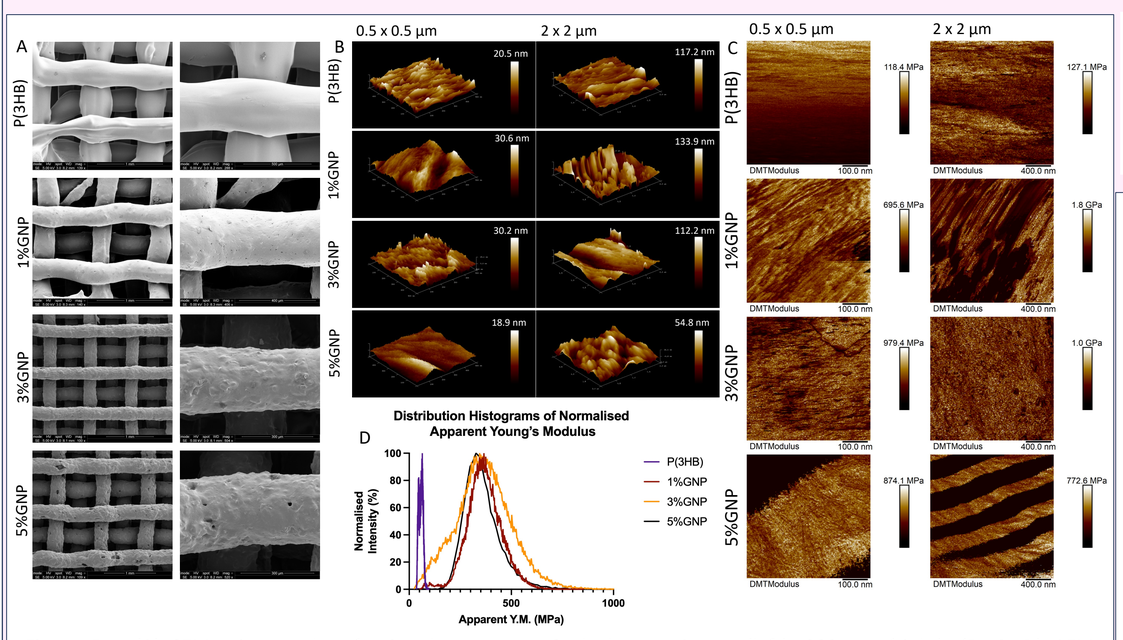


Figure 5 – (A) SEM micrographs of printed constructs. Scale bars = 1 mm (left), 300 µm (right). (B, C, D) AFM nanomechanical characterisation. (B) 3D surface topography micrographs. (C) 2D mapping of the apparent Young's Modulus. (D) Representative distribution histograms for apparent Young's Modulus of the printed constructs.

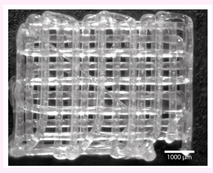


Figure 6 – Image of the melt-extruded P(3HB) construct for the SCB phase (scale bar = 1 mm).

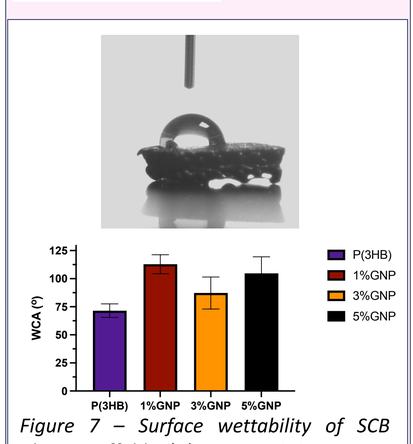


Figure 7 – Surface wettability of SCB phase scaffolds. (A) Representative image of the sessile drop on the printed constructs. (B) Data presented as mean ± SD (n=9, p <0.05).

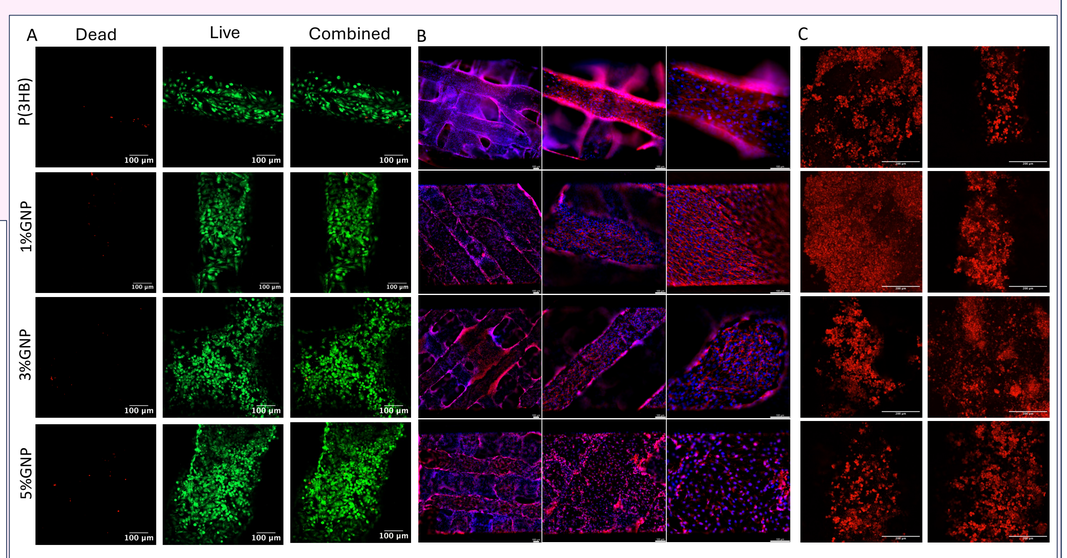


Figure 8 – Biocompatibility of 3D printed P(3HB)/GNP constructs was evaluated using SaOS-2 cells. (A) Live/Dead confocal micrographs demonstrated high cellular viability and adherence on 3D-printed struts after 7 days. Scale bar = 100 µm. (B) Fluorescent micrographs of DAPI-Phalloidin stained cells after 7 days show well-spread morphology with extended actin filaments, indicating robust cellular adhesion and cytoskeletal organisation on the composite surface. Scale bars = 100 µm. (C) Confocal micrographs of Xylenol Orange-stained mineralised nodules after 14 days of culture. Notably greater stained nodules were visually observed during imaging on 1%GNP and 3%GNP, indicating enhanced osteoblastic mineralisation. Scale bars = 200 µm.

SEM and AFM analyses (fig. 5) revealed an increase in **surface variations** proportional to GNP concentration and **apparent Young's Modulus** of P(3HB) significantly increased with GNP inclusion (fig. 5D).
Distinct **'streaked' surface patterns** observed in 5%GNP constructs (fig. 5C) were attributed to shear effects of the printer nozzle during extrusion.
Cytocompatibility was confirmed by Resazurin and PicoGreen assays, Live/Dead confocal microscopy (fig. 8A), and DAPI-Phalloidin fluorescent microscopy (fig. 8B).
Osteogenic potential was validated through evaluation of alkaline phosphatase activity and mineralisation, quantified by Alizarin Red S and visualised by Xylenol Orange staining (fig. 8C).

PHAs are highly tuneable, sustainable biomaterials capable of replicating the gradient osteochondral properties through integrated 3D printing and electrospinning to tailor the mechanical and structureal properties of the scaffolds.
Future work will evaluate electrospun scaffold biocompatibility with bovine articular chondrocytes and explore integration of the SCB and AC phases by electrospinning onto printed 3%GNP constructs.

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Conflict of Interest: D. Gregory is a founder and technical advisor for PHAsT Limited and Prof. I. Roy is one of the Directors of PHAsT Limited that develops biomedical grade polyhydroxyalkanoates (PHAs) for a variety of applications including healthcare and biomedical applications. The polymers used in this work were produced in the Roylab and not by PHAsT.