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Nanostructured Materials for Bioapplications

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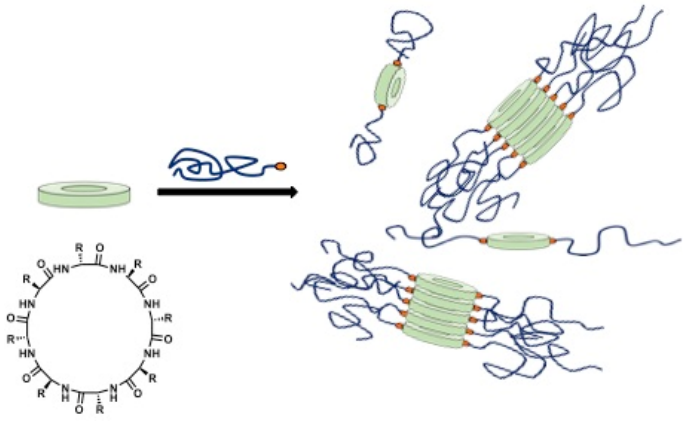
Cyclic peptide / polymer conjugates nanotubes

We describe the synthesis, characterisation and therapeutic applications of bioconjugate based on cyclic peptides covalently attached to well-controlled polymeric chains. The conjugates assemble into short nanotubes (20 to 200nm) through the stacking of the cyclic peptide motives, with functionality imparted by the polymeric chains. The resulting constructs show remarkable properties in terms of cell penetration, and *in vivo* biodistribution. We have established these systems as drug delivery vectors, enabling the efficient delivery of cancer drug and observed their therapeutic effects in vivo. We have also shown that by aligning hydrophobic chromophores along the nanotubes, we can fabricate an artificial light-harvesting system (achieving up to 95% energy transfer efficiency and a 30% fluorescence quantum yield), and ultra-bright fluorescent nanoparticles (brightness up to 12,060 m^{-1} cm^{-1} nm^{-3}). This method effectively transforms existing fluorophores into ultrabright NPs for bioimaging applications.



Mechanical and suture-holding properties of a UV-cured atelocollagen membrane for guided bone regeneration

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Introduction

Guided bone regeneration (GBR) therapy relies on barrier membranes to stabilise bone defects and prevent soft tissue infiltration. Collagen membranes are widely used for their biocompatibility and degradability. However, their mechanical fragility, excessive swelling, and rapid degradation in hydrated environments hinder their clinical utility. This study investigates UV-cured atelocollagen (AC) membranes, focusing on single (4VBC) and sequential (4VBC-MA) functionalisation, and evaluates their mechanical and suture-holding properties [1].

Experimental

The synthesis of 4VBC-functionalised AC was carried out as previously reported [2,3], with the addition of 1 wt.% polysorbate 20 and a 24-hour reaction with a 25 molar ratio of 4VBC and TEA (relative to AC lysine content). Sequential functionalisation with methacrylic anhydride (MA) was conducted similarly, using a 25 molar ratio of MA and TEA (relative to AC lysine content). Dental membranes were then prepared by UV-crosslinking with 1 wt.% Irgacure 2959 photoinitiator. Compression testing utilised a compression rate of 3 $mm \cdot min^{-1}$, and suture pull-out tests utilised a crosshead speed of 0.02 $mm \cdot min^{-1}$.

Results & Discussion

Crosslinked network architecture significantly influenced membrane performance. Sequential functionalisation with 4VBC and MA enhanced the wet-state compression modulus by twofold and reduced the swelling ratio (SR) by up to threefold, improving stability under physiological conditions.

In contrast, single-functionalised membranes demonstrated superior suture retention strength (SRS) in both dry and short-term hydrated states, with values ranging from 28–35 $N \cdot mm^{-1}$, outperforming the commercial Bio-Gide® membrane.

Scanning electron microscopy (SEM) revealed that ethanol dehydration densified the membrane microstructure, reducing porosity and enhancing mechanical properties. Atomic force microscopy (AFM) nanoindentation confirmed that sequential functionalisation increased surface elasticity and adhesivity, enhancing the mechanical toughness of the membrane.

Conclusions

Sequential functionalisation and ethanol dehydration improved compressive strength and reduced swelling, while single-functionalised membranes excelled in suturing capabilities during early hydration. These findings support the development of tailored collagen membranes to balance mechanical resilience, handling properties, and clinical performance in bone regeneration therapies.

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Amphoteric copolymers as new pharmaceutical biomaterials: synthesis, characterisation and toxicological studies

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Amphoteric polymers present a promising strategy for enhancing nasal drug delivery due to their unique dual-functional nature, possessing both acidic and basic groups. This dual functionality allows these polymers to form strong interactions with the nasal mucosa, enhancing residence time and improving drug absorption. Their pH-responsive behaviour further enables controlled and targeted drug release in the nasal cavity, offering potential solutions to key challenges in this underexplored field.

This study explores the synthesis and characterisation of amphoteric polymers via free radical homopolymerisation of [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (MEDSPA) and its copolymerisation with 2-hydroxyethylmethacrylate (HEMA). After purification through dialysis, the structural and functional properties of these polymers were analysed using various techniques. Homopolymer PMEDSPA and MEDSPA-HEMA copolymers with feed monomer ratios of 90:10, 80:20, and 70:30 were fully water-soluble. Thermal gravimetric analysis and dynamic vapor sorption techniques revealed a strong water affinity in all polymers. Toxicological assessments included the Slug Mucosal Irritancy Test (SMIT) using *Arion lusitanicus* slugs and a live-dead assay in *Galleria mellonella* larvae following polymer solution injections. Both methods showed no adverse effects, confirming the biocompatibility of these polymers. Although further research is needed to evaluate their mucoadhesive properties and optimize applications, these preliminary results highlight their potential for nasal drug delivery.

These findings provide a basis for further development, aiming to use amphoteric polymers to enhance therapeutic outcomes. By addressing existing challenges, these systems have the potential to improve patient outcomes and offer novel treatments for a range of diseases. Future research will focus on turning these promising results into practical therapeutic applications.

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Novel Electrospun Materials for the Advancement of Lateral Flow Diagnostics

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Abstract

First developed in the 1980s, lateral flow tests (LFTSs) are membrane-based diagnostics devices that are simple to use, cheap, robust, and accessible. However, their true potential was not recognised until more recently during the COVID-19 pandemic which saw LFTs used on an unprecedented scale globally with over 20 million tests used within the UK in 12 months, allowing diagnosis and monitoring to occur beyond healthcare settings. Despite this, LFTs are fraught with issues relating to sensitivity and selectivity.

Currently, casted nitrocellulose membranes are the industry standard for LFT membranes due to their low cost, mechanical strength, and high protein binding. However, nitrocellulose membranes also have shortfalls such as sensitivity to environmental factors which affect their mechanical strength, highly flammable behaviour, and batch to batch variation due to the manufacturing method. By reconsidering the materials and production method, the membrane design could significantly change LFT performance.

Manufacturing techniques such as electrospinning allow the fabrication of nanofibrous polymer materials with high porosity, interconnected porous networks, and a high surface-to-volume ratio. The porosity of the membrane governs the capillary flow of a sample/reagent through the LFT. This in turn governs the sensitivity of the LFT.

This project facilitates a step change in materials and manufacturing techniques in LFT production by investigating the use of electrospinning to fabricate polymer membranes with superior properties relating to porosity, pore interconnectivity and reproducibility that will improve the sensitivity and selectivity of membrane-based diagnostics. This approach has the potential to address existing challenges and further expand the application of LFTs in point-of-care testing.

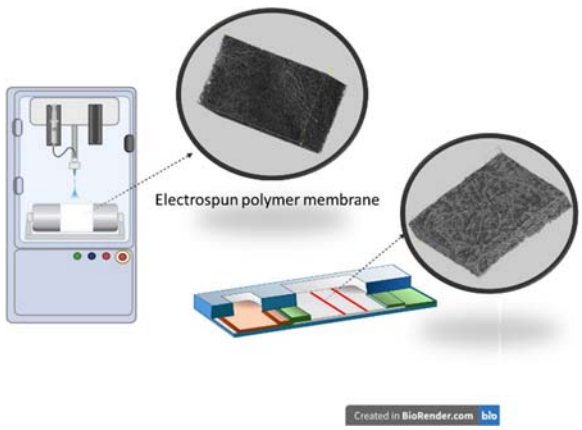

Figure 1 – Comparison of electrospun polymer membrane to commercially available nitrocellulose membranes characterized by microCT. Image stacks reconstructed from imageJ.

Bead-on-string electrospinning for the encapsulation of protein-loaded copolymer vesicles within poly(ethylene oxide) fibres

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Diseases affecting mucosal epithelia are highly prevalent and often debilitating, yet there are few dosage forms suitable for site-specific drug delivery to these tissues. A formulation containing mucoadhesive and hydrophilic polymer fibres prepared using electrospinning previously showed efficacy for the delivery of small molecule drugs to the oral mucosa.[1,2] In principle, this technology could enable the delivery of colloidal drug carriers (such as copolymer vesicles). This would greatly expand treatment options by allowing the delivery of therapeutic biopolymers (e.g. antibodies and nucleic acids), which often require carriers to enhance permeation or direct cell uptake.

Protein-loaded poly(glycerol monomethacrylate)-b-poly(2-hydroxypropyl methacrylate) vesicles were prepared using polymerisation-induced self-assembly in aqueous media with subsequent purification by size-exclusion chromatography. Electrospinning was achieved using a 2% w/v aqueous dispersion of vesicles containing 3% w/v poly(ethylene oxide) (PEO).[3] This relatively low concentration of the fibre-forming PEO polymer produced fibres with a bead-on-string morphology that contained approximately 40% w/w vesicles, as confirmed by ^{1}H NMR spectroscopy. Transmission electron microscopy (TEM) and fluorescence microscopy studies confirmed that vesicles accumulate preferentially within the beads and that each bead contains multiple vesicles. Dissolving the PEO fibres in aqueous media released the vesicles, with TEM and dynamic light scattering studies indicating that they remained intact.

Vesicles were loaded with F(ab) antibody fragments – which serve as a model therapeutic biopolymer – and encapsulated within PEO fibres. An enzyme-linked immunosorbent assay revealed that 78% of the F(ab) retained its antigen-binding functionality and remained encapsulated within the vesicles following fibre dissolution. PEO fibres containing fluorescently-labelled vesicles were applied to tissue-engineered models of the oral epithelium. This indicated successful delivery of the vesicles throughout the epithelium with minimal irritancy.

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Designing the self-assembling peptide - biopolymer bioinks for 3D bio-printing applications

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Introduction:

Gelatin methacrylate (GelMa) is commonly used in 3D bioprinting due to its ability to be crosslink under mild UV using biocompatible crosslinkers to create stable scaffolds suitable for the culture of range of cells. One of the main challenge is that GelMa is a liquid and needs to be crosslinked while printing to ensure stability of the printed structure. Here we explore the use of GelMa – self-assembling peptide hydrogels (SAPH) to design novel composite that can 3Dbioprinting and crosslinked post printing.

Materials & Method

For the purpose of the work GelMa and the self-assembling peptide KFEFKFEFKK were used to design novel composite hydrogels. First the physicochemical properties of each system was investigated using a range of techniques. Subsequently GelMa-SAPH composites were designed and their printability explored.

Result

SAPH were shown to be highly printable thanks to their shear-thinning properties. SAPH being not crosslinked the structure formed could not me handles and lifted off the printing surface. GelMa printing was challenging as crosslinking needs to be performed while printing giving a small printability window. By combining GelMa and SAPH we were able to create a composite hydrogel that could be easily printed and crosslinked post-printing to create scaffolds that could be handles and lifted from the printing surface.

Conclusion

The combination of SAPH and GelMa allowed us to created novel bioinks that could be easily printing and used to create well defined structures. We are now investigate the use of these novel materials to print cells.

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Silica mesoporous nanostar and their interaction with biological environment

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Advanced plasma surface engineering for cardiac cell manipulation

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Introduction

Cardiac tissue engineering promotes the creation of suitable in vitro environments that mimic native cardiac microenvironments for bioapplications, i.e. drug testing, disease modelling, and “heart-on-a-chip” systems. Polystyrene (PS) is widely used but appears inherently limited and necessitates surface modifications to support cardiac normal behaviour. Plasma treatments offer a versatile approach to enhance the biointerface of PS by altering its surface chemistry and morphology, thus are used in a plethora of cases. There is no systematic study however that directly compares the different plasma treatments’ effects on cardiac cells. This work addresses the above-mentioned gap in literature.

Methods

PS slides are treated using plasma in a reactive ion etcher with O_2, N_2, O_2+N_2, and Ar+N_2 gases for 20 and 30 minutes. The treated surfaces are characterised using scanning electron microscopy (SEM) for morphological analysis, X-ray photoelectron spectroscopy (XPS) for surface chemistry, and contact angle measurements (CA) for wettability. Biological assessments include culturing H9c2 cells and primary neonatal rat cardiomyocytes on the treated PS surfaces, followed by immunostaining and confocal microscopy to evaluate cell morphology, adhesion, and sarcomeric organisation. Quantitative viability assays determine the effect of each treatment on cell health and oxidative stress.

Results and Discussion

The effects of popular plasma treatments used on PS surfaces are compared to determine the optimal method for cardiac cell engineering. SEM, XPS, and CA reveal that N_2 leads to long-term hydrophilicity and a favourable nanoscale topography, significantly improving H9c2 and primary cardiomyocyte adhesion, organisation, and viability. N_2 plasma-treated surfaces support organised sarcomere structures and well-defined α-actinin, critical for cardiac function in vitro. Cardiomyocytes on N_2-treated PS exhibited elongated morphology, enhanced focal adhesion contacts, and organised sarcomeres that are essential for functional contraction. Contrarily, cells on O_2-treated surfaces exhibit a round ‘abnormal’ morphology and reduced sarcomere organisation, indicating an unfavourable environment for cardiac cell function. These findings underscore the significance of surface chemistry and nanoscale roughness in cardiac cell engineering.

Conclusion

This study demonstrates that N_2 plasma treatment is the preferred surface modification for PS in cardiac cell culture, outperforming O_2 and mixed plasma treatments in maintaining cell viability and functional morphology. These findings challenge the conventional reliance on O_2 plasma, highlighting the need for cell-specific surface engineering to create optimal biomaterial interfaces. This work advances PS-based applications in cardiac research and provides a foundation for future studies on plasma-treated biomaterials in tissue engineering.

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Spatially-resolved transfection by porous silicon-mediated optoporation

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Introduction

Engineering spatial control of gene expression through targeted nucleic acid delivery is critical for developing advanced tissue models to understand cellular interactions and biological processes. While the delivery of membrane-impermeable materials remains challenging, optoporation has emerged as a promising solution, using high-intensity laser pulses to create transient membrane pores[1]. However, traditional approaches using gold and carbon-based nanoparticles as photosensitisers face limitations due to toxicity and non-biodegradability[3]. We present biodegradable porous silicon nanoparticles as an alternative that enables spatially-resolved transfection through optoporation.

Results and Discussion

Discoid-like porous silicon nanoparticles of approximately 300 nm in size were functionalised with an azobenzene-lysine photo-switchable moiety. When coupled with a femtosecond near-infrared laser (800 nm), these nanoparticles enabled spatial-selective delivery of propidium iodide and eGFP mRNA in both 2D and 3D MCF-7 breast cancer cell models. The system demonstrated high cell viability and transfection efficiency up to 84% in 2D cultures. The versatility of this approach was further validated across multiple cell types, including human osteosarcoma cells (MG-63) and primary human dermal fibroblasts, with successful delivery and expression of GFP mRNA.

Conclusion

This approach establishes a promising platform for spatially-resolved nucleic acid delivery in both 2D and 3D cellular systems.

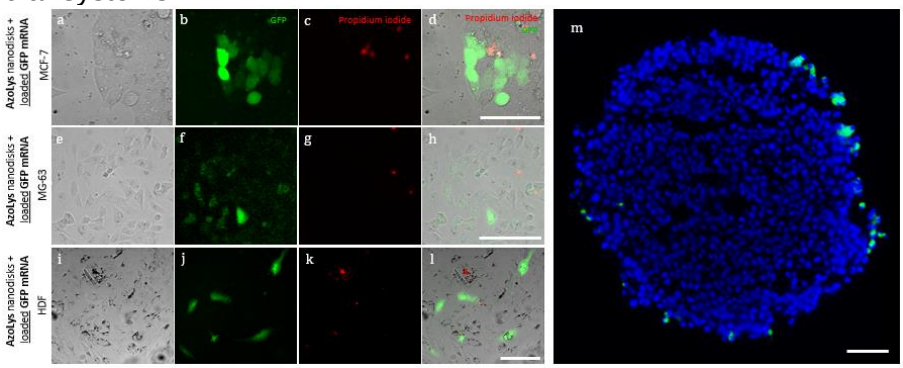

Figure 1: Spatially-resolved mRNA transfection. (a-l) microscopy images of MCF-7, MG-63 and HDF cells 24 hours following spatially-resolved mRNA transfection by optoporation. **(m)** GFP expression in the MCF-7 spheroid after localised optoporation. Scale bar: 100 µm.

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Unveiling nanometric phase formation and oxidation achieved by powder metallurgy processing routes of Zn-Mg-(Ag) alloys for bioresorbable implants

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Keywords: Zinc; bioabsorbable materials; mechanical alloying; spark plasma sintering; microstructure

Zn-based alloys have considerable potential as bioresorbable scaffolds for implants. Alloying with Mg or Ag enhances both biocompatibility and mechanical properties through precipitation strengthening and grain refinement during material production. Here we seek to advance the understanding of the precipitation mechanisms in Zn-Mg-(Ag) alloys prepared by a combination of mechanical alloying (MA), spark plasma sintering (SPS), and rapid hot extrusion for subsequent consolidation. Nanometric precipitates enriched in Mg, which have a high reactivity with oxygen, form during processing, as revealed by atom probe tomography (APT), a spatially-resolved three-dimensional mass spectroscopy technique. We provide a range of novel insights into the structure and composition of the intermetallic precipitates. The effects of microalloying additions to Zn-Mg alloys and the deformation processes on the microstructure formed and degradation behavior are often poorly characterized in the literature, leaving several knowledge gaps that our present study addresses in an effort to help guide improvements in Zn-based alloy design for bioresorbable materials.

3D PRINTING OF POLYARYLETHERKETONE (PAEK)/APATITE COMPOSITIES FOR LATTICE STRUCTURES FOR ORTHOPEADIC IMPLANTS

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Introduction

Neck and lower back pain is commonly caused by injuries or spinal disorders like degenerative scoliosis and disc disease, leading to many hospital visits globally. Spinal fusion surgery is a treatment option for these conditions. [1]. The spinal implants market was valued at $13.3 billion in 2023, with a projected 5.4% growth to 2030. [2]. While metallic implants are used for spinal fusion due to their mechanical strength, they can cause complications like stress shielding and imaging artefacts in CT scans. Polyaryletherketone (PAEK) offers a promising alternative, as it is biocompatible, strong, and can be sterilised making it a perfect candidate for healthcare applications, especially orthopedics [3]. Polyetheretherketone (PEEK) and Polyetherketoneketone (PEEK) are considered as bioinert, but Rodzen *et al.* demonstrated that adding bioactive agents such as hydroxyapatite (HA) into the matrix solves this problem and can help with aiding direct bone apposition [3]. In addition, by utilising lattice structures for orthopaedic implants this can alleviate stress-shielding, provide for enhanced surface area for the release of bioactive agents (or antimicrobial materials), and reduce imaging artefacts compared to simple solid metal implants. The aim of this work is to produce 3D printed lattice structure containing bioactive HA to enhance direct bone apposition.

Materials and Methods:

A commercial printer CreatBot (Fused Filament Fabrication) was used to print the solid samples and simple lattice structures from PEEK and PEKK. Composite samples were also 3D printed with different amounts of hydroxyapatite (HA) to try an enhance the potential bioactivity of the samples. The samples were then characterised using a range of techniques including XRD, FTIR, SEM/EDX, Micro CT, Digital Microscopy and mechanical testing.

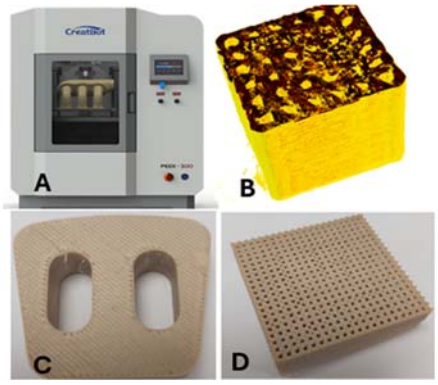

Figure 1 A) CreatBot printer B) MicroCT scan C) 3D printed spinal fusion device D) Basic Lattice

Results and Discussion

XRD and FTIR characterization highlighted that the overall chemistry of the materials did not change significantly when compared with the raw materials and filaments. Typically, the PEEK materials are semicrystalline in nature (30-45%), whereas, the PEKK can have a range of crystallinities (0-30%) depending upon the chamber temperature in the 3D printer. MicroCT results highlight the porosity, density, and morphology of the different 3D printed lattice structures. Digital Microscopy shows how the lines were printed on the 3D printer for different lattice structures and shows that the more crystalline materials are less well fused together. Compression testing highlights the difference between the solid structure and different lattice structures. Preliminary results indicate that the 3D printing conditions utilised have a significant influence on the PEEK or PEKK printed materials, namely differences in crystallinity as a result of the chamber temperature in the printer, which affects layer adhesion and ultimately the mechanical properties of the samples. If a material is too crystalline the layer adhesion can be lowered when compared to materials that were printed to be amorphous. In addition, the application of HA into composite materials can influence crystallinity also and the overall mechanical properties of the samples.

Conclusion:

These experiments show how 3D printing of PAEK/HA composites can be achieved and show significant promise. Further studies need to be conducted to understand further their mechanical, chemical and physical nature along with targeted *in vitro* characterisation.

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Design of Hierarchical Block-Copolymer Brushes for Enhanced siRNA Delivery

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Despite promising developments in the delivery of RNA therapeutics, polycationic-based delivery systems face significant challenges regarding stability and premature release, for example, due to competitive desorption associated with the formation of biomolecular coronas or interactomes in blood and in the cytosol, respectively [1].

Surface-initiated polymer brushes of unique high densities have shown to enable high binding capacities through deep infiltration of small siRNA, with sustained delivery and prolonged knockdown (KD) efficiencies [1-4].

Here, we explore strategies to enhance the stability of these delivery systems by engineering their physico-chemical and architectural features. We first designed a library of tertiary and quaternary amine-bearing polymer brushes via ATRP and investigated structure-property relationships through their RNA binding capacity, cellular internalisation, and KD performance using a combination of surface plasmon resonance, immunostaining, and flow cytometry assays. Selected binding chemistries were then used to synthesise block-copolymers with poly(N-isopropylacrylamide) (PNIPAM), where PNIPAM provides protection via its collapsed conformation at physiological temperature.

Our data revealed that binding stability strongly correlated with the brush chemistry, where polymers with lower pKa showed premature RNA release and/or reduced cellular internalisation. Notably, quaternisation emerged as an effective strategy to modulate RNA-polymer interactions and enhance transfection efficiency. Competitive binding assays demonstrated enhanced RNA retention with block-copolymer brushes compared to single binding blocks, in the presence of strongly anionic competitors. When delivered to human umbilical vein endothelial cells (HUVECs), block-copolymer brushes showed improved long-term knockdown of PECAM-1 expression. These findings provide new insights into the rational design of polymer brush-gene delivery systems as an attractive and readily adaptable platform for systematic study of complex mechanisms in gene therapy applications.

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Leveraging supramolecular polymeric biomaterials for regenerative medicine strategies

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University of Aveiro

Lysine-Rich Lipopeptides: Biosurfactants and Bioactive and Model Colloidal Systems

Prof Ian Hamley

University of Reading

The conformation and self-assembly of two pairs of models of lipidated tripeptides in aqueous solution was probed using a combination of spectroscopic methods along with cryogenic-transmission electron microscopy (cryo-TEM) and small-angle x-ray scattering (SAXS). The palmitoylated lipopeptides comprise C_{16}-YKK or C_{16}-WKK (with two L-lysine residues) or their respective derivatives containing D-lysine (k), i.e., C_{16}-Ykk and C_{16}-Wkk. All four molecules self-assemble into spherical micelles which show structure factor effects in SAXS profiles due to inter-micellar packing in aqueous solution. Consistent with micellar structures, the tripeptides in the coronas have a largely unordered conformation, as probed using spectroscopic methods. The molecules were found to have good cytocompatibility to fibroblasts at sufficiently low concentrations, although some loss of cell viability is noted at the highest concentrations examined (above the critical aggregation concentration of the lipopeptides, determined from fluorescence dye probe measurements). Preliminary tests also show antimicrobial activity against both Gram-negative and Gram-positive bacteria.[1] Small-angle x-ray scattering (SAXS) was performed on concentration series of the four lipopeptides to investigate the micelle shape and structure from the form factor and to probe inter-micellar interactions via analysis of structure factor. The structure factor enabled the inter-micellar potential to be computed based on the Hayter-Penfold model for charged colloids with screened Coulomb interactions. The critical micelle concentration (CMC) was obtained consistently from surface tension and electrical conductivity measurements and compared to the CMC from fluorescence probe experiments. Atomistic molecular dynamics (MD) simulations were performed to probe micelle structure and molecular packing and conformation within the micelles.[2] This revealed distinct features of molecular conformation for the two tryptophan-based lipopeptides which both sample extended β-sheet conformations more significantly than the two tyrosine-based lipopeptides. The aromatic residue adjacent to the lipid chain was also found to influence the conformation of the C-terminal lysine (or D-lysine) residues which sample extended α-helical conformations. These results provide unprecedented insight into the structure and interactions of lipopeptide micelles which constitute model self-assembling colloidal systems, and which have considerable potential for future applications as biosurfactants and bioactive materials.

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Influence of End-Residue on the Properties of Self-Assembled β-Sheet-Forming-Peptide Hydrogels

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Introduction:
Peptide hydrogels have emerged as promising biomaterials due to their ability to self-assemble into nanoscale structures and form hydrogels through non-covalent interactions. Their tunability makes them suitable for many biomedical applications such as tissue engineering, drug delivery and 3D-bioprinting [1]. This study investigates the impact of hydrophobic and hydrophilic modifications at the C-terminus of the parent peptide KFEFEFKFK on hydrogel's properties, focusing on self-assembly, gelation, and mechanical behavior. By incorporating phenylalanine (hydrophobic) and lysine (hydrophilic) residues, four new peptide sequences were designed: KFEFEFKFK**F** (F), KFEFEFKFK**K** (K), KFEFEFKFK**FK** (FK), and KFEFEFKFK**KF** (KF).

Materials and Methods:
Peptides self-assembly and gelation was characterized across varying pH and concentration. Phase diagrams were constructed to study gelation behavior, and structural insight was gained through TEM, SAXS, and FTIR. Hydrogel's mechanical properties were assessed using oscillatory shear rheometry.

Results and Discussion:
FTIR, TEM and SAXS results indicated that hydrophobic modifications (F, FK, KF – on additional F) enhanced β-sheet formation and promoted denser fiber network formation resulting in stable gelation across a broad pH range. The position of the end residues and the orientation of their side chains had a significant impact on the fibers' edge properties and fiber−fiber interactions. Fibers with hydrophilic terminal residue (KFEFEFKFK, K, and FK) showed a reduced tendency for lateral fiber association, leading to weaker gelation [2], while fibers with hydrophobic terminal residue (F and KF) exhibited stronger aggregation behavior depending on the orientation of the end residue side group. Rheological analysis revealed that all hydrogels were shear-thinning with more hydrophobic peptides leading to higher storage moduli and faster recovery following shear.

Conclusion:
Through this work, we demonstrated the importance of terminal residues in this peptide family on fiber edge properties and the association/crosslinking propensity of the fibers formed. The latter having a direct impact on the overall physical properties of the hydrogels including their mechanical resilience following high shear (i.e.: injection). The ability to design and fine-tune the properties of hydrogels formed by these peptides is critical for their application in the biomedical field whether as cell niches or as in-vivo delivery vehicles for cells and/or drugs [3],[4].

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3D printing of hybrid polyphosphate coacervate gels for bone scaffolds manufacturing

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Introduction

The increasing in age population and prolongated life expectancy, there are also an increase in bone-related disease, such as osteoporosis. Bone tissue has the ability to regenerate, however, large defects can delay or impair healing[1]. 3D printing is often used to manufacture complex scaffolds aimed to mimic the native tissue. After implantation, the scaffold is designed to help regeneration of the damaged tissue by supporting adhesion and growth of bone cells and facilitating the flow of fluids[1,2].

In this work, we have designed a novel ink for 3D printing of scaffolds for bone regeneration.

Discussion

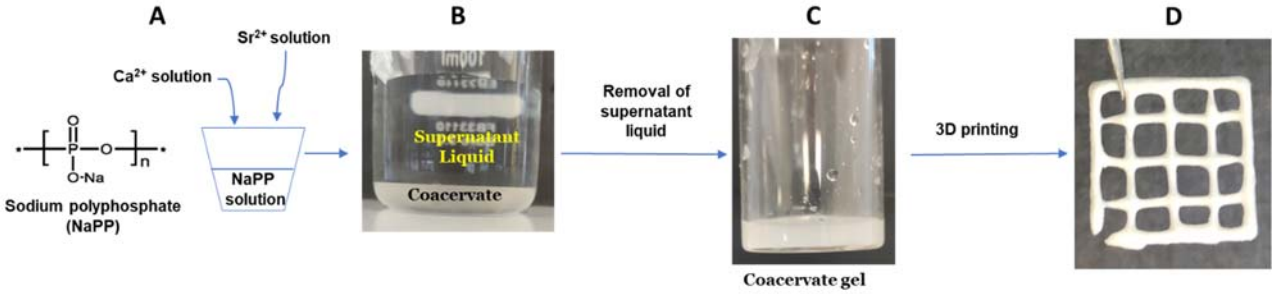
The ink is based on hybrid polyphosphate coacervate gels containing strontium and an amount of a shear-thinner polymer (ratio polymer: polyphosphate 3:1 w/w). Upon drying the scaffold would have a composition very similar to that of the native bone and facilitating its regeneration[3,4]

Addition of strontium has been found to simultaneously affect improve osteoblast proliferation and inhibit osteoclast cells[5] Polyphosphate coacervate gels are obtained by slowly adding a 2M aqueous solution of Ca^{2+} and Sr^{2+} to a 4M aqueous solution of sodium polyphosphate (NaPP) (Fig 1 A).

After 24h, a phase separation occurs (coacervation, Figure B) with the coacervate gel forming at the bottom and the supernatant at the top. Once isolated (Figure C), the coacervate gel is mixed with the polymer in different ratios to produce a suitable ink for the printing. The printed scaffold was then either directly freeze-dried (Figure D) or first cross-linked and then freeze-dried.

Conclusion

Rheological properties of the inks and the mechanical properties of the scaffolds were assessed to optimise compositions of the inks with the final aim to obtain the best bone regeneration scaffold



Figures 1 – Synthesis of a hybrid polyphosphate-scaffold via the coacervation process

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RENOVITE®: A Novel Injectable Clinical-Grade Nanoclay for Targeted Biomedical Applications

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Introduction: Nanoclays (NC) are layered silicates with high aspect ratios and permanent negatively charged surfaces. Their inherent negative charge and cation-exchange capacity enable efficient interactions with biomolecules, creating opportunities for regenerative medicine and drug delivery applications.[1] Certain synthetic hectorites such as Laponite form delaminated dispersions in water and generate gels due to charge interactions between the particles. These colloidal gels are highly sensitive to changes in ionic concentration, pH, and the presence of organic compounds. RENOVITE®, a proprietary analogue of synthetic hectorite clay, has been designed with an optimized magnesium-to-lithium ratio[2], which enhances clay gelation in physiological fluids compared to the commercial synthetic hectorite, Laponite™ (XLG).

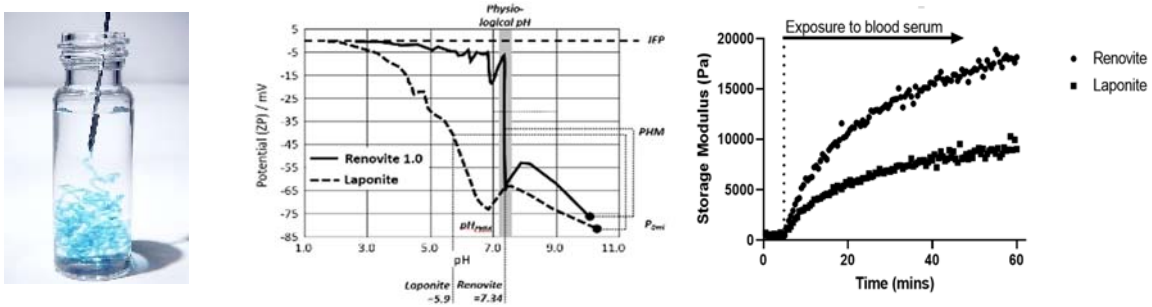

Figure 1: Injectable NC (left), zeta potential measurement against pH sweep (center), and storage modulus (Pa) of nanoclay gels (right).

Results and Discussion: RENOVITE® was synthesized via a controlled hydrothermal process. Elemental composition was confirmed using ICP-OES. Structural properties were characterized through XRD and FTIR, confirming the hectorite analogue. SAXS and TEM were used for particle size analysis. SAXS revealed the disc-like structure of the nanoparticles, with an average radius of 15.22 ± 0.60 nm and a thickness of 1.43 ± 0.12 nm. Zeta potential measurements using a Stabino™ II device with acid titration demonstrated a 50% decrease in negative zeta potential for RENOVITE® between pH 8 and pH 5, with particle agglomeration occurring at a physiological pH of 7.33, in contrast to XLG, which agglomerated at a lower pH of 5.9. Polyelectrolyte titration to determine the surface charges of NC, revealed a slightly high charge of XLG as compared to the RENOVITE®. Rheometric analysis revealed an associated increase in storage modulus of 17144.0 ± 427.7 Pa following serum exposure, signifying enhanced gel stiffness under physiological conditions (Figure 1).

Conclusion: RENOVITE is a clinical-grade injectable nanoclay gel that provides a stable, localised matrix at physiological pH, offering an effective solution for targeted biomedical applications.

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The development of ionotropically gelled alginate to mimic the mechanical properties of skin

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Introduction

Hydrogels are a widely used class of materials in the biomedical field and are deployed across a range of applications due to their tunable physical and chemical properties. Given that human tissues are mechanically anisotropic, there is a desire to create hydrogels that can exhibit defined mechanical properties and heterogeneity found in tissues. In the case of skin, full thickness placements should ideally be designed to match the mechanical properties of distinct tissue layers[1]. Alginate-based hydrogels have many advantages, but their crosslinking can be heterogenous and so there is a need to enhance the gelation process through the addition of components or the use of secondary cross-linking steps[2]. Unlike rapid gelation, slow gelation methods provide a mechanism to control the timing of gelation and to ensure homogeneity, allowing injectable and moldable formulations while maintaining structural integrity[1].

Method

GAP hydrogels were developed using alginate (A) and gelatin (G) as primary components. Alginate solution was gradually added to a stirred gelatin solution to produce a homogeneous GA hydrogel mixture (v/v). Poly (ethylene glycol) diacrylate, PEGDA, (P) was then incorporated at final concentrations of 5–10% (w/v), followed by a $CaCO_3$-GDL mixture at a different 1:2 molar ratio. Our experimental procedure optimized the ratios of $CaCO_3$ and Glucono-δ-lactone (GDL), allowing gelation to occur under nearly neutral pH conditions. The mixture was poured into cylindrical molds and incubated at 37°C, 5% CO_2 for 15 min to promote initial ionic crosslinking. Secondly, further crosslinking was achieved by exposure to UV light (360–420 nm) for 2 min, a process that was carefully optimized to avoid cell damage. Then the samples were incubated overnight to ensure complete gelation.

Result and Discussion

The mechanical properties of the varying concentration of GAP hydrogels were evaluated by uniaxial compression tests using cylindrical samples. The stress (σ)-strain (ε) curves revealed nonlinear elastic behavior similar to the mechanical response of human skin.
At the low strain ($\varepsilon < 15\%$), the Young's modulus (~100 kPa) closely aligned with that of dermal tissue. At higher strain ($15\% < \varepsilon < 60\%$), the hydrogel exhibited strain-stiffening behavior, with an average tangent modulus exceeding 1 MPa. To further characterize this mechanical performance, hyperelastic models such as Mooney-Rivlin, Ogden, and Fung models were applied to the experimental dataset by using MATLAB (MathWorks, Inc., Natick, MA)[3]. These models demonstrated a high degree of accuracy ($R^2 > 0.99$), validating the GAP hydrogel's capacity to replicate the nonlinear mechanical properties typical of biological tissues.

Conclusion

This study presents a two-step cross-linked hydrogel system designed for skin tissue engineering, combining biocompatibility, tunable gelation, and tissue-like mechanics. The formulation shows great potential for advancing engineered tissue scaffolds in regenerative medicine.

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Glycan polymers and beyond

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Characterisation of an Electroactive hydrogel actuator and its biomedical applications

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Introduction:
Smart materials can change shape upon exposure to an external stimulus. Here we created a smart material that can change shape upon exposure to an electrical current (1). Commonly known as ionic electroactive actuators (EAPs), these materials can change shape upon exposure to an electrical current. They carry this current through redox reactions. An important advantage of using Ionic-EAPs is the low voltage required for actuation to occur. Here, we have created a smart material containing 2-acrylamido-2-methylpropane sulfonic acid (AMPs) and instead of Acrylamide, it cross linked it to Polyethylene Glycol Diacrylate (PEGDA) to produce a 3-dimensional actuating polymer, which can have a variety of different biomedical applications such as drug delivery systems, soft robotics, and medical devices (1,2).

Results and Discussion:
PEGDA was used as the secondary polymeric chain since the AMPs was unable to create a 3-dimensional structure. Mechanical testing of the hydrogel was performed to determine the effect change in cure time has on the stiffness of the material. Further actuation testing was performed on the material to determine the effect cure time has on the speed of actuation. Reaction schemes have been produced of the free radical addition polymerisation reaction and have been verified using FTIR analysis. Finally, ISO standard cytotoxicity testing has been performed on the hydrogel, to determine cytotoxicity of the material.

Conclusion:
We have successfully created a biocompatible and smart polyanionic material that can actuate upon exposure to an electrical current of 5V and 0.1A. Interestingly, as the cure time increases, although, this increases the stiffness of the material, more AMPs monomers get recruited, and so creating a faster actuation.

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A cold atmospheric plasma-composite hydrogel delivery system for anti-cancer drugs

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We have developed a composite hydrogel drug delivery system, that, when activated with cold atmospheric plasma (CAP), releases drugs in therapeutic concentrations. [1] The hydrogel system, comprised of super absorbent sodium polyacrylate particles (PAA) buried within a cryo-crosslinked polyvinyl alcohol (PVA) matrix, can be loaded with a wide range of cationic molecules. The basis of the system is the coulombic interaction between positively charged amine groups on drug molecules and the carboxylate groups in the PAA particle, allowing a drug payload to be entrapped within the PAA (Figure 1a). The application of CAP, which comprises of a wide range of reactive oxygen and nitrogen species (including H_2O_2, •OH, NO, NO_2^- NO_3^-, $ONOO^-$), as well as electric fields, charged species and photons, effects controlled release of the drug from the PAA particles, whilst simultaneously delivering beneficial CAP species (Figure 1b); CAP has previously been shown to enhance the efficacy of drugs. [2, 3]

In this presentation, we demonstrate that the technology works with a wide range of drugs including anti-microbials, anti-inflammatory agents and chemotherapeutic drugs. We show that cisplatin (and other chemotherapeutic drugs) can be loaded into a composite hydrogel and delivered on demand by application of CAP to the gel, as well as the effect of co-delivery of cisplatin with CAP against 2D and 3D (spheroid) head and neck cancer cell lines. We show that CAP allows delivery of molecules across the epidermis, facilitating deeper tissue penetration of drugs that are otherwise difficult to deliver topically due to poor skin penetration.

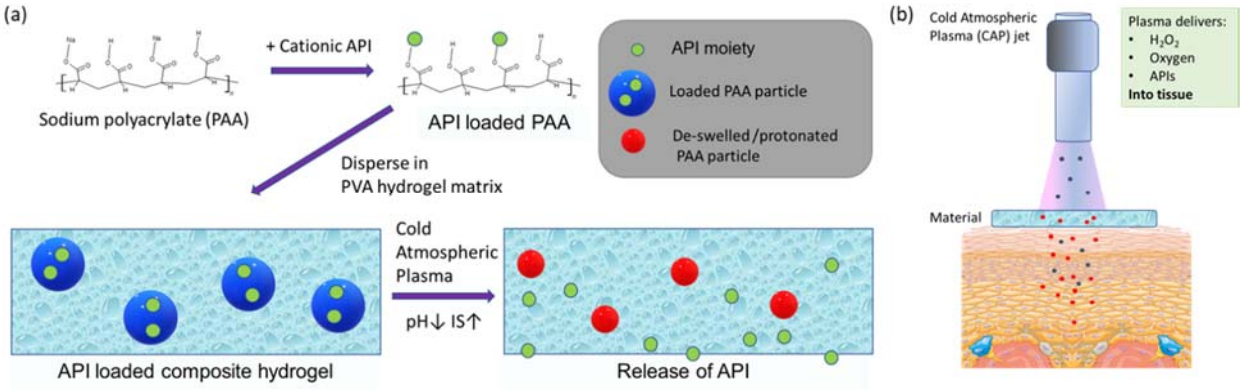

Figure 1: (a) Schematic of the composite hydrogel drug system; (b) Application of CAP releases and drives drugs deep into tissue alongside beneficial CAP components.

This work was supported by EPSRC grant EP/V00462X/1.

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Nanoparticle label-free tracking technique as an indirect hydrogel characterization platform

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Introduction

Hydrogels are used extensively in biomedical research due to their tuneable properties, biocompatibility, and controlled degradation profiles. When combined with biological factors they have regenerative capacity and can also be used as carriers for drugs and nanotherapeutics, in addition they are currently used as a range of synthetic tissue analogues enhancing in vitro experimental models. Due to the wide-ranging applications and heterogenous tuneable properties, levels of characterisation must provide not only bulk material properties, but also an understanding of how changes in phases inherent to the hydrogel affect the behaviour of any added agents/particles within the local (nanoscale) environment. Available techniques for the characterisation of hydrogels at the nanoscale have limitations for low viscous hydrogels and involve tedious sample preparation and expensive equipment. Here we aim to use a label-free nanoparticle tracking technique as a passive hydrogel characterisation tool at the nanoscale, using an optical inverted microscope, by applying the optical phenomena of caustics[1].

Methodology

Gold nanoparticles (AuNP) of 100 nm of diameter were used across all samples as standard, these were added in each of the solutions/hydrogels to reach a constant concentration of 10^7 particle/mL. The AuNPs were tracked in different glycerol solutions and silicone oils to perform a correlation curve between the nanoparticle's experimental diffusion coefficient and the viscosity of the media. These values were then used to i) characterise local viscosities in heterogeneous hydrogels based in agar-hyaluronic acid as in vitro vitreous humour models[2] and to ii) characterise the phase temperature transition in Pluronic F127 20% w/v a thermosensitive hydrogel for drug delivery purposes. The nanoparticles were tracked over time using a label-free technique, based on the optical phenomena of caustics, on an inverted optical microscope. The tracking data was analysed with the ImageJ TrackMate plugin to obtain experimental values of the diffusion coefficient.

Results and discussion

The high correlation (R^2= 0.96) of experimental values of diffusion coefficients and viscosity of the media proved the effectiveness of using diffusion as an indirect predictor of localized viscosity in the vicinity of the nanoparticle's location, validating the technique as a tool for characetrising viscosity at the nanoscale. The label-free nanoparticle tracking platform uncovered the heterogenicity of the tested hydrogels, the use of the previously mentioned standard curve enabled the prediction of the viscosity values of each of these characterised localized environments. Furthermore, the tracking of the nanoparticles at specific temperatures (ranging from 20˚C to 40˚C) in Pluronic F127 at 20% successfully characterized the $T_{sol\text{-}gel}$ transition at 28˚C, in agreement with rheological data. This technique proved to enable not only the characterisation of these features in real-time but also to better understand the nanoparticle-hydrogel interaction.

Conclusion

The use of the label free nanoparticle tracking technique has proven to be an easy-to-use, inexpensive and accurate technique to characterize nanoparticle diffusion in complex environments, permitting a better understanding of nanoparticle hydrogel interaction but more importantly to characterise at the same time hydrogel features at the nanoscale.

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Self-Assembling Peptide Hydrogels: Moving towards Sustainable 3D Liver Models in Drug Discovery

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The reliance on animal models and animal-derived matrices in preclinical drug discovery faces increasing criticism due to ethical concerns and limited translatability to human physiology [1]. Drug-induced liver injury (DILI) remains a significant challenge, being one of the major causes of drug withdrawals, often due to hepatotoxicity undetected in animal studies [2]. Similarly, Matrigel, a widely used animal-derived matrix, suffers from variability and undefined composition, limiting its suitability for consistent and predictive in vitro models [3]. This study aims to develop a reproducible, xeno-free peptide hydrogel for supporting hiPSC-derived hepatic organoid culture and improving hepatotoxicity prediction.

We utilized self-assembling peptide hydrogels with the sequence EEFKFEFKFEE, which demonstrated >90% viabilityin 3D HepG2 cultures over 35 days. The hydrogel supported the formation of spheroid clusters (~50 μm) without necrotic cores, with polarized structures confirmed by F-actin localization. Functional assays indicated sustained albumin and urea secretion, highlighting liver-specific activity. Acetaminophen LC_{50} values in peptide hydrogels were significantly lower than in Matrigel, indicating improved hepatotoxicity sensitivity.

To explore its potential as a Matrigel replacement, the hydrogel was tested as a substrate for hiPSC culture. IKVAV-functionalized hydrogels exhibited the highest cell adhesion, followed by GFOGER, while other motifs showed minimal or no cluster formation. Preliminary hiPSC cultures on functionalized hydrogels demonstrated strong attachment, embryoid body formation, and maintained pluripotency markers (OCT4, TRA-1-60), showing high potential for differentiation into hepatic organoids.

This study introduces a novel xeno-free peptide hydrogel as a reproducible, defined and potentially alternative to Matrigel. The hydrogel supports long-term HepG2 spheroid viability and improves hepatotoxicity detection. Additionally, functionalization with IKVAV and GFOGER promotes hiPSC adhesion and differentiation potential, advancing the development of reliable, animal-free models for drug discovery.

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A tuneable hydrogel platform based on platinum-containing polymeric arsenicals

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Platinum and arsenic (e.g. cis-platin, As_2O_3) have been used extensively in modern medicine due to their strong anticancer and antimicrobial activity. However, intrinsic and acquired resistance are major problems associated with Pt^{II} and (in)organic arsenicals are notoriously toxic. Consequently, the development of alternative chemistries and delivery strategies for Pt and As containing molecules and materials is of high importance. Here, polymeric arsenical scaffolds (**P1** - **P4**) containing 2, 4, 6 and 8 mol% of As-functionalised acrylamide monomer (AsAm) have been mixed with Pt^{II} (from K_2PtCl_4, [As] : [Pt] = 1 : 1) giving rise to hydrogels (**P1-Pt** – **P4-Pt**). Crucially, hydrogels are only formed when AsAm is present in the polymer scaffold and Pt^{II} is added, with the rate of gelation (**P4** > **P3** > **P2** > **P1**) and subsequent properties of the hydrogels dependent upon the AsAm feed. The nature of the Pt-polymer interaction has been thoroughly investigated by ^{1}H and ^{195}Pt NMR spectroscopy as well as FT-IR, SEM, XPS and potentiometric titration suggesting that crosslinking of **P1** – **P4** occurs primarily via coordination between oxygen atoms of the pendant arsenic acid ($R\text{-}AsO_3H_2$) group and Pt^{II} (K_{f2} = 1.1 x 10^5 M^{-2}). Secondary non-covalent interactions (e.g. chain entanglements, π-π interactions and solvent-polymer hydrogen bonding) are proposed to provide further structural integrity and stability. The combination of weak Pt-polymer coordination bonds and non-covalent interactions furnishes soft (G' < 350 Pa) but relatively strong hydrogels, that reach an upper maximum load at 4.5 MPa (500 N, 30 secs, 10 % deformation). The Young's modulus increased from 9 - 14 kPa across **P1-Pt** – **P4-Pt** with **P4-Pt** exhibiting the greatest stiffness and **P1-Pt** the best elasticity. The hydrogels also demonstrated potential self-healing properties, with free-standing hydrogels yielding comparable moduli (G', G'') and yield points (τ_y) by rheology obtained after sequential incision and healing steps. The nature of the crosslinking promotes deep penetration of water into the loosely crosslinked networks. Equilibrium swelling ratios of 2425 % and 1875 % were measured for **P3-Pt** and **P4-Pt** respectively, whilst **P1-Pt** and **P2-Pt** reached > 1000 % swelling before losing structural integrity. Finally, preliminary antimicrobial evaluation conducted via disk diffusion and resazurin bacterial cell viability assays indicates that **P4-Pt** is active against Gram-negative (Uropathogenic *Escherichia coli*, *UPEC*) and Gram-positive (*Staphylococcus aureus*, *S. aureus*) bacterial strains. Overall, the combination of previously reported polymeric arsenical scaffolds with Pt^{II} results in the formation of crosslinked networks generating soft, strong, self-healing hydrogels with tuneable stiffness and elasticity that possess antimicrobial activity.

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Smart Polymeric Microneedles for Non-invasive Transdermal Drug Delivery and Electrochemical Biosensing

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KAUST University

Enzyme Responsive Hydrogels For Personalised Drug Delivery

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Introduction: Disease recovery and survival rates following peritoneal tumour resection surgery are poor,[1] largely due to the long-term inefficacy of post-surgery chemotherapies.

This project aims to deliver chemotherapeutics via an implantable controlled release hydrogel depot. The hydrogel provides an extracellular enzyme triggered release of the chemotherapeutic in a prolonged and sustained manner at the tumour site.

Elevated β-glucuronidase levels have been reported in primary tumours and metastases. Therefore, the growth of the tumour following surgery will lead to an increase in extracellular enzyme concentrations, which we aim to exploit to trigger the release of an antitumour drug via an enzymatically degradable linker from a hydrogel depot.

Methods: Hydrogel design: A PEG based hydrogel network will incorporate enzyme cleavable linkers to facilitate the release of the antitumour drug, Paclitaxel (PTX) (Fig 1). Hydrolytic stability of the gel will be evaluated for a range of crosslinkers tested using NMR analysis and viscosity sensitive fluorophores.

Results: Five of the eight synthetic steps towards the enzyme responsive hydrogel have been completed. Preliminary hydrogel stability results for a number of crosslinkers have been obtained showing promising insights.

Conclusions: Significant progress towards the development of the β-glucuronidase responsive hydrogel has been made. Preliminary data for the hydrolytic stability of the 4-arm PEG gel shows significant differences in hydrogel degradation rates for the crosslinkers tested.

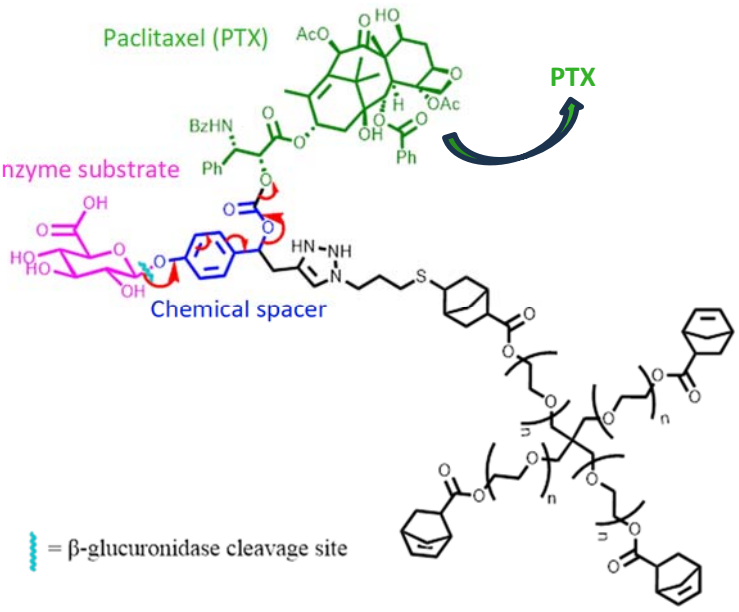

Figure 1. Representation of 4-arm PEG hydrogel components and β-glucuronidase triggered release of PTX.

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Nanoneedle-Based Electroporation for Efficient Manufacturing of Human Primary Chimeric Antigen Receptor Regulatory T-Cells

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Introduction

Regulatory T cells (Tregs) are promising for treating autoimmune diseases and preventing transplant rejection due to their ability to suppress excessive immune responses. Genetically engineering Tregs with chimeric antigen receptors (CARs) can enhance their specificity and efficacy[1]. However, the current reliance on viral transduction for CAR-Tregs production presents significant challenges, including immunogenicity, insertional mutagenesis risk, and high costs. Nanoneedle arrays, consisting of high-aspect-ratio nanostructures, are highly effective in delivering genetic materials into hard-to-transfect cells without inducing toxicity[2]. This study introduces a scalable nanoneedle electroporation (nN-EP) platform designed for GMP-compatible transfection of HLA-A2-specific CAR plasmids into primary human Tregs.

Results and Discussion

nN-EP achieved a transfection efficiency of 43.07%, outperforming viral transduction at MOI 1 by twofold. Additionally, nN-EP transfection maintained high cell viability at 87.33%, compared to 55.4% with bulk electroporation. Importantly, nN-EP did not alter the phenotype and proliferation of Tregs, meeting the expansion requirements for clinical applications. The generated CAR-Tregs exhibited targeted immunomodulatory capabilities, effectively suppressing the proliferation of effector T cells in an antigen-specific manner when co-cultured with an HLA-A2^{+} B-lymphoblastoid cells.

Conclusion

nN-EP platform provides a reliable and effective method for manufacturing primary human CAR-Tregs and paves the way for broader clinical applications in immune therapies.

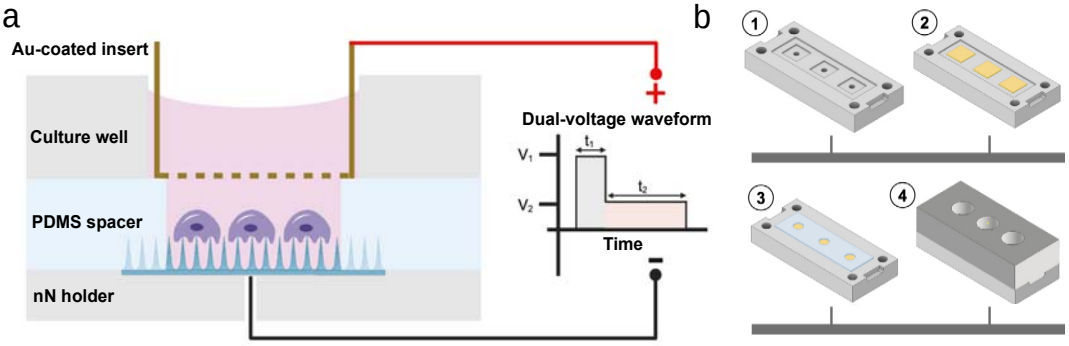

Figure 1: Nanoneedle electroporation of Tregs. (a) Schematic illustration of the nN-EP-system setup. Not to scale. (b) Stepwise assembly of the nN-EP-system: Stage 1 shows the bottom nN holder; Stage 2 shows the nN chips positioned within the holding areas; Stage 3 shows the PDMS spacer layered over the nN chips; and Stage 4 shows the fully assembled nanoneedle electroporation well.

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Nanoneedle biopsy for nondestructive temporal lipidomics of tissue

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Abstract: Spatial lipidomics, which measures the lipids profile from tissue sections, has provided invaluable insights into the molecular mechanisms underlying various metabolomic processes[1]. However, these processes are inherently dynamic, with the lipidome landscapes evolving over time in tissue. Current spatial lipidomics methods, typically using fresh-frozen tissue sections, provide only static snapshots of these dynamic processes, limiting our ability to observe and track the temporal dynamics of lipids within the same tissue.

Nanoneedles, arrays of vertical, high-aspect-ratio nanostructure, have been widely characterized as a versatile platform for transmembrane delivery and intracellular sensing[2, 3]. Nanoneedles can probe individual biomarkers or small biomarker panels[4], by sampling intracellular content in a non-perturbing matter. Moreover, through the repeated sampling of the same cell or regions of tissue, nanoneedles can longitudinally monitor their molecular profiles[5].

This work engineers a nanoneedle biopsy platform for spatiotemporal omics, specifically targeting lipidomics in the mouse brain. We have established an automatic nanoneedle biopsy platform capable of interfacing with tissues to generate molecular replicas consistently and reliably. These molecular replicas preserved both the molecular composition and their spatial information from the tissue, enabling desorption electrospray ionization mass spectrometry imaging (DESI-MSI) for lipidomics on nanoneedles thus preserving the tissue specimen. We first demonstrated that molecular replicas could reproduce the lipid distribution present within proximal tissue sections, accurately outlining the key anatomical regions, such as gray matter, white matter, and tumor lesions within the mouse brain. For spatiotemporal lipidomics, we further investigated treatment responses by characterizing changes in lipid composition within live glioblastoma-bearing mouse brain samples.

Keywords: Spatial lipidomics, temporal dynamics, nanoneedles, mouse brain

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Identification of key drivers of liposome drug release

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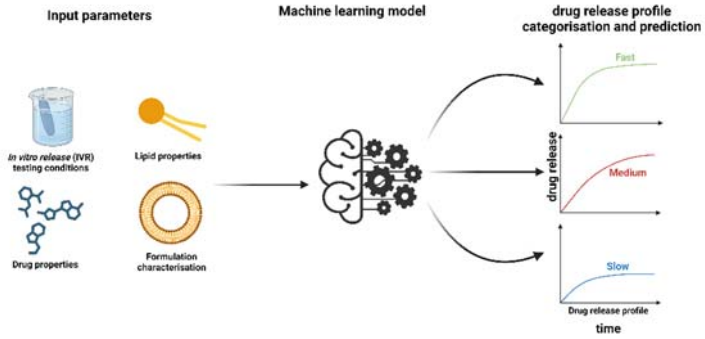
Drug release from nanomedicines is a complicated process.[1] During the product development of nanomedicines, *In vitro* (IVR) release tests are employed to understand product performance.[2] Due to the complexity of nanomedicine products, there is a gap in the fundamental understanding of key drivers of drug release from nanomedicines. This gap causes a bottleneck in the development of nanomedicine products. This research aims to evaluate the underlying factors controlling drug release from liposomes, to determine the extent to which drug and lipid properties, formulation characteristics and IVR testing conditions (feature inputs) modulate drug release. A comprehensive database was compiled from 34 research papers, resulting in 272 *IVR* profiles. 7 Machine Learning (ML) classifiers are screened to establish relationships between feature inputs to fitted kinetic model parameters describing drug release profiles. The ML training process is interrogated via SHapley Additive exPlanations (SHAP) analysis to reveal key parameters influencing types of drug release behaviour. Using interpretable ML, the user choice of IVR testing conditions is found to have greater influence on release kinetics than other feature inputs. A Random Forest Classifier model is identified as the best performing, to predict the type of drug release behaviour given feature inputs. The model demonstrates 9-10 correct predictions out of 13 unseen examples. Compared to a random baseline, there is a significant improvement in balanced accuracy score from 0.30 to 0.71. Using ML models to unravel complex multivariate processes such as drug release from liposomes can help identify key parameters driving distinct types of release behaviour. This enables priorisation of experimental and/or formulation parameters to alter to modulate drug release. A benchmark performance is established for the classification of liposome release kinetics, given feature inputs. This research takes a step towards the development of a predictive tool to accelerate the design of nanomedicine IVR tests.

Graphical abstract



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Simple & Smart: Minimalistic Design Approach for the Development of Peptide Nanomaterials

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Infection Wars: A New Hope

Professor Raechelle A. D'Sa

University of Liverpool

Antimicrobial resistance (AMR) represents a significant threat to global health and has been caused by overuse and misuse of antibiotics in the healthcare and agricultural sectors. AMR is responsible for 1.27 million directly and 5 million associated deaths annually worldwide and by 2050 it is predicted this number could jump to 10 million deaths/year. These dire statistics emphasise the urgent need to develop alternative approaches. New antimicrobial agents such as nitric oxide, antimicrobial peptides and antimicrobial metals hold significant promise in the ongoing fight against AMR. Although promising, the ability to deliver these antimicrobial agents to the site of an infection in a controlled and sustained manner is where the challenge lies. Polymeric advanced material platforms are promising vehicles for the efficient delivery of antimicrobial agents due to their tailorable chemical compositions, microstructures and biological properties. Our lab has developed these advanced polymeric platforms for treatment of biomedical infections such as: contact lens hydrogels to target microbial keratitis; electrospun dressings, emulsion gels and hydrogels to treat skin wound infections; polymeric nanoparticles to treat respiratory infections; and electrospun and 3D printed patches to treat dental infections. The combination of novel antimicrobial agents with effective delivery systems represents a new hope as we move into the post-antibiotic era.

Design and evaluation of cationic antimicrobial polymers

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Abstract

The worldwide spread of antibiotic resistance due to the overuse of antibiotics urges the need for novel less prone to resistance antibiotics.[1] Antimicrobial peptides (AMPs) are small polypeptides that contribute to the innate immunity system. Their activity and selectivity towards microorganisms are both attributed to their amphiphilic structure.[2] However, AMPs instability and high production costs limited their clinical development as prospective antibiotic.[3] Synthetic antimicrobial polymers (SAMPs) were introduced as promising antibiotics that mimic the amphiphilic structure of AMPs combining hydrophobic and cationic polymers in various ratios. This design is challenging as increasing the positive charge will decrease the overall hydrophobicity of the polymer and vice versa.[3,4] Previous work in our group focused on the synthesis of SAMPs that mimic the common amino acids found in AMPs, e.g. arginine, lysin and leucin, to provide similar amphiphilicity structure and activity.[5,6]

In this work, diblock copolymers of the apolar monomer N-isopropyl acrylamide (NIPAm) and either the cationic amino ethyl acrylamide (AEAm), dimethyl amino ethyl acrylamide (DMAEAm), or trimethyl amino ethyl acrylamide (TMAEAm) were synthesized with 30 and 70% cationic monomer content in various length (DP) and conformation (diblocks and triblocks). The copolymers were obtained by RAFT polymerization and evaluated for activity and hemocompatibility (hemolysis and Hemagglutination).
The polymers minimum inhibitory concentration (MIC) according to the standard Clinical Laboratory Standards Institute (CLSI) broth microdilution method (M07-A9-2012) were determined against gram positive and negative bacteria *Staphylococcus aureus* (*S.A*) and *Pseudomonas aeruginosa* (*P.A*), respectively.

The results showed no activity of the DMAEAm or TMAEAm copolymers at the highest concentration tested (512 µg/ml). AEAm diblocks had activity at 64 µg/ml against *S.A.* for structure $pNIPAM_{15}$-*b*-$AEAM_{35.}$ The same trend was observed against *P.A*, although the polymers were less effective by one-fold. Intriguingly, pDMAEAm homopolymers were active at 64-32 µg/ml against *S.A* for pDMAEAm above DP40.

The results so far support the hypothesis of the importance of the cationic charge for activity but also emphasis the importance of the type of charge as primary ammonium was superior to quaternized ammonium copolymers. However, the effect of the apolar monomer (NIPAm) seems to be of less importance for DMAEAm copolymers.

All the copolymers showed no hemolytic activity, suggesting low toxicity to mammalian cells. Although hemagglutination was observed in the active copolymers, these copolymers can still be employed for topical applications.

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Chlorhexidine Digluconate Epoxy Resin: A Durable and Potent Antimicrobial Coating to Eliminate Microbial Contamination and Disease Transmission

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Introduction

Disease transmission is encouraged by the rapid spread of microorganisms. The majority of surfaces harbour a vast range of bacterial, fungal and viral pathogens which can be transmitted to individuals upon direct contact (1). One increasingly more popular method of disease prevention aims to eliminate the microbial reservoirs present on surfaces and other frequently touched fomites. Chlorhexidine digluconate (CHDG) is a potent antimicrobial and has been utilized across medical and dental industries for the prevention and treatment of infections due to its high bacteriocidal, fungicidal and viricidal properties (2). Previously we showed that our cured CHDG epoxy resin coating exhibits excellent antimicrobial efficacy against a variety of microorganisms, including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*, as well as demonstrating high durability and a good distribution of the active agent throughout the surface. This research expands on previous findings by exploring the long-term stability of the cured coating, its antimicrobial efficacy when applied to real-life surfaces and the chemistry behind the surface regarding incorporation of CHDG into the epoxy resin facilitated by X-ray Photoelectron Spectroscopy (XPS) and 3D OrbiSIMS analysis.

Results and Discussion

12 months after curing, the CHDG epoxy resin demonstrated highly potent antimicrobial efficacy against *E. coli*, a ubiquitous, causative pathogen of nosocomial- and community-acquired bloodstream, urinary and gastrointestinal tract infections (3). Further, following artificially-accelerated aging using UV to degrade the surface, the antimicrobial coating demonstrated 100% bacteriocidal efficacy compared to control epoxy resin, highlighting that this coating can retain high potency against problematic pathogens over a long duration. Finally, surface characterisation of the coatings may suggest that the chlorine present in the CHDG structure is binding to the epoxy resin, potentially indicating a new, undefined antimicrobial mechanism of action.

Conclusions

Our novel CHDG epoxy resin coating demonstrates high antimicrobial efficacy against a variety of clinically-relevant pathogens. It exhibits excellent long-term durability and stability in addition to retaining bacteriocidal potency after 12 months. Finally, evidence suggests that the CHDG present in the epoxy resin may be exploiting a new mechanism of action following evidence of antimicrobial-epoxy resin binding.

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Design of Peptide Hydrogels for Ocular Drug Delivery

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Introduction: Wet macular degeneration (wet-AMD) is a progressive eye disease caused by abnormal leaky blood vessels, resulting in loss of central vision. Formation of these new blood vessels is driven by increased vascular endothelial growth factor (VEGF) and, therefore, treatment focuses on intravitreal injection of anti-VEGF drugs. Such injections are currently administered every 4-6 weeks, bringing several drawbacks such as patient discomfort, risk of infection and non-compliance as high as 60% within the first 2 years[1]. Consequently, there is an urgent need for strategies to sustain release of anti-VEGF therapeutics and decrease treatment frequency. Self-assembling peptide hydrogels have been identified as promising drug delivery systems as they can be designed as injectable, biocompatible and biodegradable[2]. They are capable of trapping small molecules within their cross-linked polymer network and slowly releasing them through diffusion, hydrogel swelling and over time, degradation[3]. This research aims to reduce the frequency of wet-AMD treatment by designing peptide hydrogels as a local drug delivery system to extend the time at which the anti-VEGF drug Avastin remains within a therapeutic range. Here, we vary the chemistries of the peptide sequences and explore how this influences hydrogel physical behaviour and consequently their ability to prolong the long-term release of the anti-VEGF Avastin.

Methods: Peptide hydrogels with six different primary peptide sequences were formed by pH triggered β-sheet self-assembly. Peptide hydrogels (35mg/mL) loaded with Avastin (6.25mg/mL) were characterised and compared by rheology, FTIR and swelling ratio for their suitability as drug delivery systems. Drug release was monitored using the Bradford Assay. Biocompatibility was investigated in vitro with immortalised ARPE-19 cells under standard culture conditions. Proliferation and viability were quantified using Pico Green and LDH assays respectively.

Results: Ions present in Avastin's formulation buffer were found to affect gelation and mechanical properties of the hydrogel when compared to the pure peptide hydrogels, likely due to charge screening. We demonstrated that the peptide was still able to self-assemble into fibers with characteristic β-sheet secondary structure following drug encapsulation. The drug loaded hydrogel was also shown to have shear-thinning properties, making it suitable as an injectable depot. The peptide sequence is known to dictate fiber-fiber interactions, influencing the extent of bundling and network crosslinking. This is demonstrated by the six hydrogels exhibiting a range of swelling and degradation profiles which related to their mechanical properties. The Avastin release profiles varied between the hydrogels, ranging from 11%-54% release within the first 7 days. All 35mg/mL hydrogels sustained release for at least 7 weeks in vitro. Our hydrogel did not decrease cell viability or impact proliferation and is therefore non-cytotoxic.

Conclusion: Overall, this work demonstrates the potential of peptide hydrogels as a drug delivery vehicle for more targeted and temporal delivery of Avastin in the treatment of wet-AMD.

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Function and Delivery of Antifibrotic Polysaccharides

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Introduction

Fibrotic conditions account for nearly half of all deaths in industrialised countries. Scarring in the eye can lead to blindness, and impaired healing following burns can lead to significant disfigurement and impairment. Conditions such as epidermolysis bullosa lead to progressive scarring of the skin and mucosa, triggered by the motions of everyday life. There is thus an urgent need for antifibrotic therapies that are not only efficacious, but also affordable, and more easily brought to market.

Results

Natural polysaccharides were studied for their antifibrotic activity. *In vitro* assays showed that carrageenan and alginate were able to inhibit fibroblasts from transdifferentiating into myofibroblasts, shown by a significant downregulation of alpha smooth muscle actin. Of these, the iota carrageenan subtype was shown to be most effective, and acted in a concentration dependent manner. Optical techniques were then developed to probe the mechanism of this action, which is hypothesised to be the sequestration of the profibrotic transforming growth factor beta 1 by the polysaccharide. Iota carrageenan was formulated into a structured fluid through ionic cross-linking under shear, to create high-polymer density particles within an interstitial phase. This material was highly viscoelastic, but also readily sprayable for ease of application due to its discontinuous microstructure.

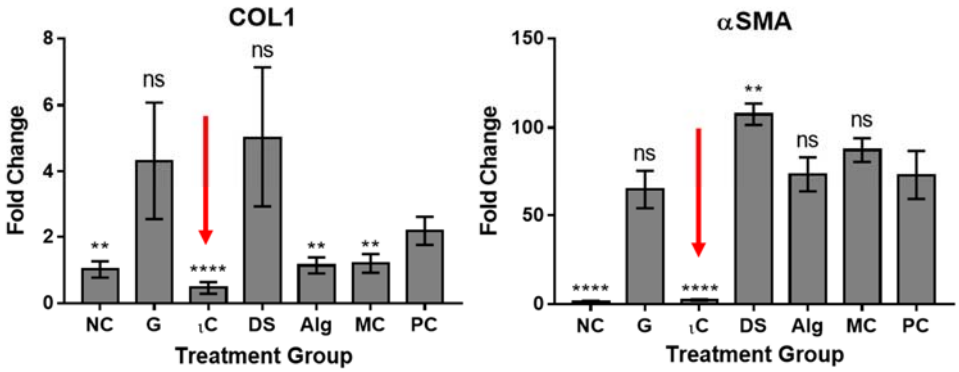

Figure 1: Downregulation of fibrosis-related genes by natural polysaccharides

Discussion

Natural polysaccharides are abundant, low cost, and have a long history of use in food, cosmetics and healthcare, making them an attractive option as an economical therapeutic with a simplified route to market. This study has identified promising candidates which may act to prevent scarring by sequestering profibrotic factors, though this mechanism needs further scrutiny. The particular physicochemical properties of iota carrageenan which give it this particular functionality, compared to other polysaccharides with similar structures and functional groups, may further elicit this mechanism, and also allow design of a 'perfect' polymer for this function. The ability to create healthcare materials where a single component provides both the therapeutic and structural properties, so-called 'self-delivery', is an enticing proposition for translation.

NO-releasing liposomal formulations for the treatment of respiratory infections

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Abstract

The endogenously produced free radical nitric oxide (NO) has attracted much attention for its broad-spectrum multi-mechanism antimicrobial activity [1]. However, due to its chemical instability and very short physiological half-life, exogenous and tuneable NO chemical donors have been designed to prolong its rate of delivery [1]. Herein, we encapsulated NO donors into a variety of stable stealth liposomes with ~ 55-85% encapsulation efficiency. NO-loaded liposomes were developed to further tune and protect premature NO-release kinetics in physiological environment and enable lung drug delivery via nebulisation. The NO-loaded-liposomes were characterised with a hydrodynamic size of ~ 100-115 nm (polydispersity index of ~ 0.13-0.2) and smooth spherical morphology (Figure 1A and 1B), neutral surface charge (ζ-potential ~ −2 mV) and a sustained release profile of NO over 24 h in PBS monitored by a NO chemiluminescence analyzer. The NO-releasing-liposomes exhibited significant ($P < 0.01$) antimicrobial efficacy against planktonic methicillin-resistant *Staphylococcus aureus* (MRSA) and the gram-negative multidrug-resistant *Pseudomonas aeruginosa* bacteria compared to the free NO-donor, particularly at lower tested concentrations and at the longer 24 h incubation time-point. Notably, the most optimal formulations demonstrated a complete kill for the MRSA and a > 5 log reduction for the *Pseudomonas aeruginosa*. Uptake of DiI-labelled NO-liposomes by bacterial pathogens showed increased uptake in gram-negative bacteria and capsule knockout strains as compared to gram-positive bacteria (Figure 1C). Moreover, neutrophils showed high uptake (~ 100%) at all tested doses of NO-liposomes among other primary nasal mucosal cells (Figure 1D). The cytocompatibility of the liposomal formulations were also assessed against the NL20 human bronchial epithelial cells and all optimal formulations demonstrated > 70% cell viability. Overall, our results show the NO-releasing liposomal formulations were highly effective in eradicating resistant respiratory bacterial strains which hold significant promise as an alternative to the use of antibiotics for respiratory infections.

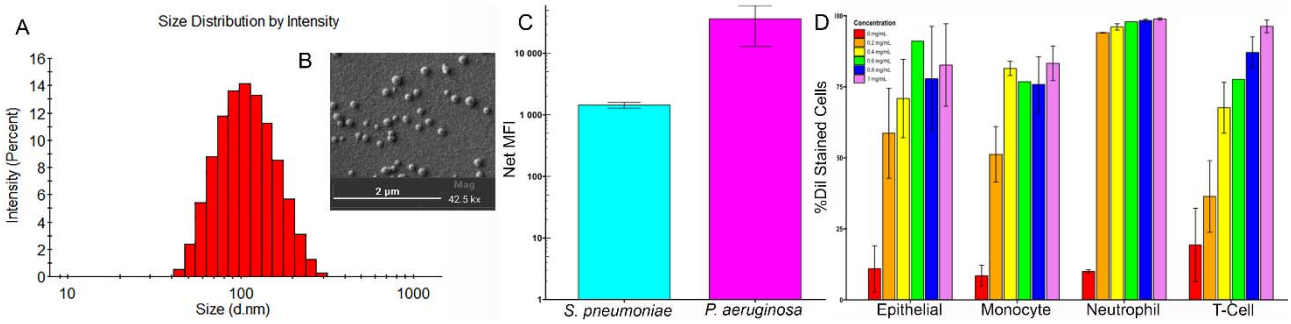

Figure 1. A) Intensity weighed hydrodynamic size distribution of NO-liposomes as assessed by dynamic light scattering. B) Cryo-SEM image of NO-liposomes showing spherical smooth morphology. C) Uptake quantitation of fluorescently labelled NO-liposomes in gram-positive and gram-negative bacteria by flow cytometry. D) Liposome uptake by primary nasal mucosal cells.

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Tuning the gelation temperature of injectable thermogels

Prof Theoni Georgiou

Imperial College London

Development of Enzyme-responsive Nanogels for Treating Inflammatory Diseases

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Abstract

Inflammatory diseases often persist despite treatment with anti-inflammatory drugs, which fail to resolve underlying inflammation. Targeting inflammation resolution offers a promising strategy to prevent further tissue damage. Annexin A1 (ANXA1), a pro-resolving mediator with potent anti-inflammatory properties, has rarely been investigated for its delivery. Here, we developed matrix metalloproteinase (MMP)-2-responsive nanogels (NGs) to achieve controlled protein release at inflamed sites. These NGs demonstrated favourable characteristics, including sizes of 100–200 nm, slightly positive surface charge (~10 mV), high protein loading (>10%), encapsulation efficiency (>70%), excellent biocompatibility, and efficient cellular uptake. Based on this platform, we synthesised Cathepsin B (CTSB)--responsive nanogels for intracellular drug delivery. GFP mRNA-loaded CTSB-responsive NGs exhibited higher transfection efficiency in M1 macrophages induced by LPS and IFN-γ, compared to uninduced RAW264.7 macrophages, indicating their potential for controlled drug release at inflamed sites. In addition, double-layered NGs with dual enzyme responsiveness were designed to facilitate both intramedullary and extracellular drug delivery, incorporating an MMP-2-responsive outer layer to deliver ANXA1 and a CTSB-responsive inner layer for the delivery of gene-editing tools (e.g., the CRISPR/Cas9 system and mRNA). Preliminary studies confirmed the successful formation of these bilayer NGs, which are anticipated to enable precise spatiotemporal drug release and offer effective novel therapies for treating inflammatory diseases.

Redox-responsive nanogels facilitate *in vitro* and *in vivo* RNA delivery

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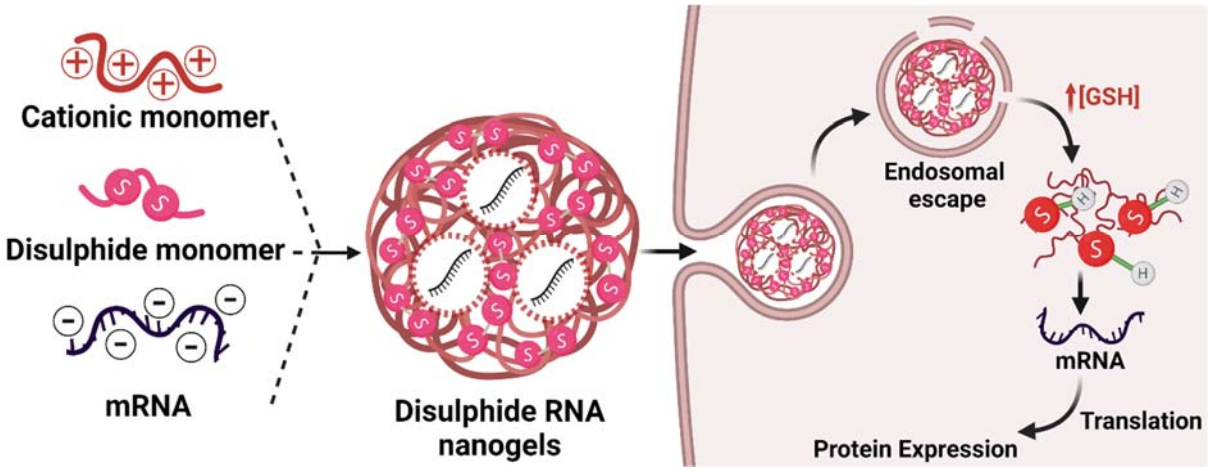
RNA delivery using RNA-encapsulating nanoparticles holds transformative potential for applications in treatment, diagnostics, and biosensing. Lipid nanoparticles (LNPs) have showcased the promise of RNA nanocarriers in stabilising RNA and improving in vivo delivery, though their cellular uptake remains suboptimal. In contrast, polymeric delivery vehicles demonstrate considerable chemical versatility, resulting from the ability to conjugate multiple functional groups onto the same polymer backbone, thereby endowing them with desirable properties. Nanogels, a class of polymeric nanoparticles, possess extensive cargo-loading capacities and exceptional biocompatibility. Functionalised with stimuli-responsive moieties, multi-functional nanogels can enable spatiotemporal control over RNA release with enhanced biological specificity. We report the encapsulation of diverse RNA types within disulphide nanogels, that can exploit the [glutathione (GSH)] gradient between extracellular ([GSH]: 2-20 µM) and intracellular ([GSH]: 1-10 mM) environments, to mediate cytosolic RNA delivery. Bioassays confirmed their ability to achieve controlled mRNA release, sustaining prolonged protein expression comparable to commercial reagents. Further, the work presents the endolysosomal escape evaluation of these nanocarriers, thereby elucidating the mechanism by which cellular delivery is achieved. Moreover, we present the *in vivo* biodistribution profile of actively targeted, disulphide-based nanogels for the first time, extending opportunities for specific disease interventions via highly precise mRNA delivery.



Magnetic nanogels for combined hyperthermia and chemotherapy of prostate cancer

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Chemotherapy patients suffer severe side effects from the medications and hyperthermia can be an invasive treatment[1]. In recent years, clinical trials have demonstrated that hyperthermia can enhance the action of chemotherapy drugs[2] as cancer cells are susceptible to heat[1]. Combining the two therapies allows to establish synergistic actions and minimise their individual drawbacks.

The superparamagnetic iron oxide nanoflowers (IONFs) are known to have excellent heating abilities[3]. Nanogels are biocompatible 3D networks of crosslinked polymer chains. Nanogels encapsulate water, cargo and provide drug-carrier properties for smart delivery. In this work, we are developing a combined hyperthermia and chemotherapy treatments for prostate cancer with iron oxide magnetic nanogels[4].

The nanogel synthesis is performed with an *in situ* noncovalent electrostatically driven template polymerization around the IONFs. The presence of nanogels is proven though size and zeta potential measurements with the dynamic light scattering machine. The IONFs have d_{DLS}= 70 nm and ζ = -23 mV, while the nanogels have d_{DLS}= 408 nm = 363 nm and ζ = +12 mV.

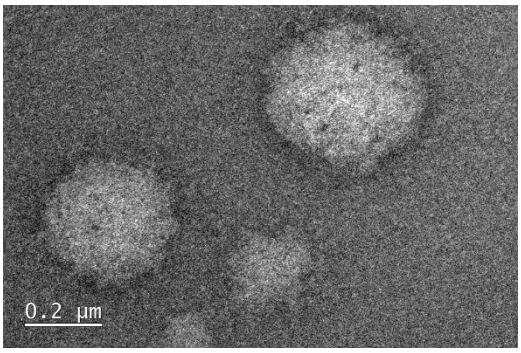

Figure 1 Magnetic nanogels under TEM, negative stain, 20k.

The nanogels were observed after negative stain treatment under transmission electron microscopy (TEM), with d_{TEM}= 363 nm (Figure 1). The iron content inside the nanogels was 79.50% (determined by inductively coupled plasma - optical emission spectrometry ICP-OES). Finally, the magnetic hysteresis loop was recorded with the superconducting quantum interference device (SQUID); the results showed IONFs still superparamagnetic. In a magnetic field with amplitude 16 kA/m and frequency 350 kHz, the specific absorption rate (SAR) was 2698 W/g_{Fe} and the intrinsic loss parameter (ILP) was 9.88 nHm^2/kg_{Fe}. These parameters exceed the state-of-the-art magnetic nanogels (SAR of 47 W/g_{Fe} and ILP of 2.8 nHm^2/kg_{Fe})[5].
The magnetic nanogels demonstrated excellent cell viability up to 100 µg/mL.

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Development of Inhaled Therapeutic Polymeric Nanoparticles for the Treatment of Respiratory Infections

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Introduction:
Respiratory diseases and infections are a significant global public health concern, ranking third and fourth among the leading causes of death worldwide (WHO), with lower respiratory infections being the leading communicable cause of death, resulting in 2.6 million deaths in 2019.[1] Treatment of bacterial respiratory infections is increasingly problematic due to the rise of multi-drug resistant bacteria. Nitric oxide (NO) is a promising antimicrobial alternative to antibiotics. NO effectively kills a broad range of microorganisms by disrupting cellular functions its multimechanistic action makes bacterial resistance unlikely. However, as NO is a gas, delivering it to infection sites is challenging. This project aims to develop polymeric drug delivery vehicles to encapsulate NO donors for inhaled applications.

Methods:
These polymers were selected for their low toxicity and readily available monomers. Both linear and hyperbranched versions of two cationic polymers, each with distinct degradable backbones, were synthesized in anhydrous solvents for high yield and purity. The reactions were optimized to produce high molecular weights (10-30 kDa). Two hyperbranching monomers were used to explore the impact of structural changes. The polymers were then modified and nitrosylated with an in-house NO-donor, allowing them to store high quantities of nitric oxide. Their antimicrobial activity was tested against *Staphylococcus aureus* (*S. aureus)* and *Pseudomonas aeruginosa* (PA01).

Results:
Characterisation of the in house synthesised polymers was performed using, 1H, 13C, 13C DEPT, COSY, HSQC, HMBC nuclear magnetic resonance spectroscopy (NMR), FTIR and gel permeation chromatography (GPC). NO release was directly measured in PBS under varying conditions (light/ dark, EDTA, pH) by chemiluminescence.

Both linear polymers exhibit high payloads of nitric oxide, releasing concentrations of 250-300 μM mg^{-2} and 300-350 μM mg^{-2}, respectively, over a 24 hour period. Antimicrobial testing indicates efficacy against both gram positive and negative bacteria. Linear polymer 1 shows complete kill at 5 mg against S. aureus and 7.5 mg against PA01 after 24 hours. Whilst linear polymer 2 achieves complete kill at 5 mg after 24 hours against *S. aureus* and PA01. In nutrient poor conditions, both linear polymers demonstrate complete bacterial kill at 2.5 mg against S. aureus and PA01.

Conclusions:
We have synthesized linear and hyperbranched polymeric drug delivery carriers covalently bonded to NO donor molecules. These polymers demonstrated controlled, sustained NO release, effectively killing both gram-positive bacteria and gram-negative bacteria over 24 hours in both nutrient rich and poor conditions. Future work includes aerosolization tests, coculture antimicrobial efficacy testing, and cell cytocompatibility analysis for further development.

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Plasma activated hydrogel release of Polymyxin B for wound disinfection

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Cold atmospheric plasma (CAP) is an ionised gas that is generated at atmospheric pressure and below 40°C. It produces a wide range of reactive oxygen and nitrogen species (including H_2O_2, •OH, NO, NO_2^-, NO_3^-, and $ONOO^-$) as well as other physical components, such as electric fields, charged species, and photons. CAP has been shown to be beneficial for a wide range of medical indications, including wound disinfection and healing, cancer, and inflammatory skin conditions [1-3]. Most of these treatments involve the direct use of CAP on tissue, but there is growing interest in combining CAP with drugs due to the potential synergistic effect which could enhance the chosen drug's efficacy [4,5].

Plasma activated hydrogel therapy (PAHT) is a recent advancement in the use of CAP which has been developed for a variety of conditions including fungal infections, vitiligo, and cancers [1]. In PAHT, a drug loaded hydrogel is placed between the plasma jet and the target. It can then be triggered to release drugs in therapeutic concentrations by the application of CAP. These drugs are co-delivered deep into the tissue alongside the beneficial components of plasma (Figure 1). This technique also offers additional advantages over the direct use of CAP on tissue because the hydrogel screens out the potentially hazardous short lived/high energy components of plasma (such as •OH) while allowing long lived species to pass through (e.g. H_2O_2, O_2) and reduces the dehydration of tissue [6].

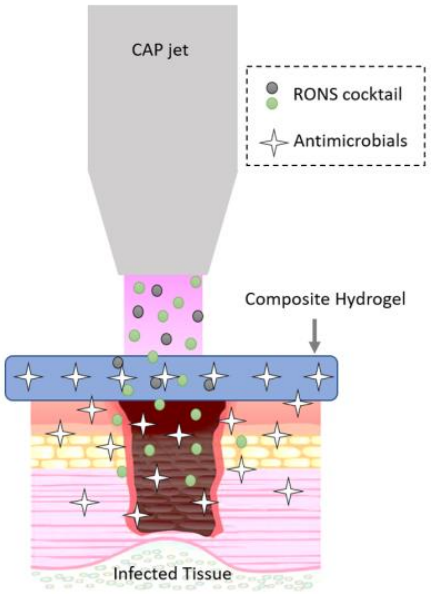

Figure 1 – An illustration of infected tissue being treated with plasma activated hydrogel therapy (PAHT) [6].

Here we show the loading and release of the polypeptide antimicrobial Polymyxin B from a sodium polyacrylate (PAA)/poly vinyl alcohol (PVA) composite hydrogel. The PAA swelling ratios were calculated for a range of polymyxin B solution concentrations before the preparation of polymyxin B loaded PAA/PVA composite hydrogels. These hydrogels were cryo-crosslinked using a freeze-thaw methodology. They were then treated with a helium plasma jet to trigger the release of the drug from the PAA particles (Figure 1) and incubated at 37°C for 24 hours prior to the collection and measurement of the released drug.

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Development of Peptide-Chitosan Hydrogel Composites for Wound Healing Applications

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Introduction: Infections continue to pose significant challenges in the treatment of wounded tissue. In recent years, wound dressings made from natural polymers have gained popularity due to their anti-inflammatory and antibacterial properties.[1] The materials used for wound dressings must possess moisturizing properties, biodegradability, and mechanical strength. Accordingly, Peptide hydrogels are being considered due to their ability to meet these requirements.[2,3] The microbial resistance and mechanical properties of peptides can be enhanced by other polymers like chitosan. Chitosan is widely recognized as a natural antimicrobial and biodegradable polymer, particularly in therapeutic applications like wound healing.[4] In this study, we optimized a peptide/chitosan hydrogel to develop an antibacterial hydrogel with improved mechanical properties. The physical and chemical characteristics, release properties, and antibacterial activity were thoroughly investigated.
Method: First, chitosan dissolved in a weak acid solution (0.08 M HCl) and stirred for 24 hours. Then, peptide powder was added to the chitosan solution in different weight ratios (1:1, 1:2, 1:4, and 1:8) to evaluate how these concentrations affect the final properties of the composite. The mixture was stirred until a uniform composite was obtained. Mechanical and viscoelastic properties were assessed using shear rheology. FTIR and UV-VIS spectroscopy were performed to confirm interactions between peptide and chitosan. The antibacterial properties of the composite were tested against E. coli and Bacillus strains.
Results: The results demonstrate that peptide-chitosan composite hydrogels exhibit enhanced mechanical and antibacterial properties. FTIR and UV spectroscopy show the interaction between peptide and chitosan. Peptide hydrogels containing chitosan exhibited greater storage modules regarding rheology. Furthermore, the peptide-chitosan hydrogel showed a significant reduction in bacterial growth.
conclusion: The peptide-chitosan hydrogel exhibited significantly enhanced antibacterial properties compared to peptide hydrogel and chitosan hydrogel alone. Future work will focus on further exploring its antibacterial properties and optimizing the hydrogel for potential use in wound healing.

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key words: Peptide, Chitosan, Hydrogel, Antibacterial activity, Wound healing

OXYGEN-MODULATED BIOMIMETIC *'BREATHING'* TISSUE FROM PHOTO-CROSSLINKED PROTOCELLS

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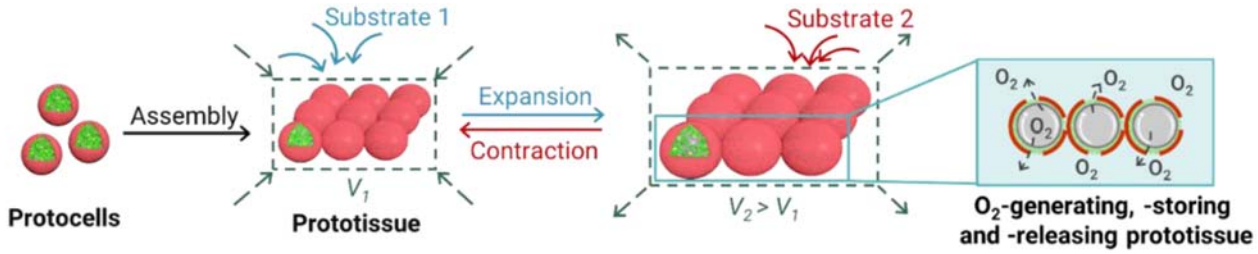
Introduction: Biomimicry involves assessing design principles of biological systems and recreating materials with the ability to emulate functions and capabilities similar to their biological equivalents. Living tissues comprises multicompartment constructs, with specialized cells of various chemical compositions. Despite efforts in producing artificial compartments (protocells), due to the structural and chemical complexity, fabricating functional synthetic tissues (protissues) with defined microarchitectures are still a challenge. Inspired by the hierarchical structure and dynamic deformations of the lung alveoli, this research aims to construct a *'Breathing'* prototissue that possess similar structure and function to the alveoli.

Results and Discussion:



Utilising bottom-up biomimetic designs, photo-crosslinkable protocells have been developed by utilizing a complexation between two biopolymers. They can encapsulate various biomolecules and microparticles, serving as functional programmable protocells. We have employed antagonistic activity between enzymes within the protocells to render rhythmic oxygen (O2) bubble formation and consumption leading to the reversible expansion and contraction of the protocells, which can be switched on and off. Subsequently, the photo-crosslinkable protocells have been arranged and crosslinked forming self-standing interconnected multicompartment prototissues with defined unique complexities, significantly, without the need for additional supports. The assembly of these specialized protocells results in the formation of prototissues capable of exhibiting collective expansion and contraction, which is similar to those observed in the alveoli. The *Breathing'* tissue have been proven to be used as O2 generating, storing and releasing units as they can retain the generated O2, and the O2 can be slowly released into a hypoxia environment. Moreover, communication, using O2 as a signal, between the protocells within the prototissues, and between the prototissues and living cells could be achieved.

Conclusion: Since the photo-crosslinkable microcapsules are made from biopolymers, the prototissue could be potentially integrated in tissue engineering applications such as restoring damaged tissues by supplying O2. The programmable properties of the photo-crosslikable protocells make them suitable for the development of functional protocells and prototissues. Importantly, by combining a photo-crosslinkable component into the microcapsules, our strategy provides a pathway for creating self-standing prototissues incorporate diverse regions emulating in living tissues, that enable the recreation of rudimentary cellular behaviors, while tackling issues in the fields of synthetic biology and tissue engineering, such as the construction of matrix-free self-standing prototissues with organized microarchitectures.

Design and synthesis of self-assembling peptide hydrogel materials for spinal cord injury repair

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Imperial College London

Spinal cord injury has significant impacts on patient quality of life and is associated with a significant economic cost of £1.12 million per patient over their lifetime. To date, there are no curative treatments for this condition and patients suffer from a multitude of health and societal issues. Neural tissue engineering approaches that use bioinstructive biomaterials to support and guide repair and regeneration of critical spinal cord tissue may offer a solution. As spinal cord injuries are typified by complex, patient-specific lesion geometry and challenging anatomical locations, injectable hydrogel materials have been found to exhibit many key desirable features. In particular, self-assembling peptide hydrogels can be chemically and mechanically tuned to present appropriate cell guidance cues for proliferation, differentiation and axonogenesis. Moreover, conductive substrates and biomaterials have been demonstrated to improve the development and establishment of neural circuits. It is envisioned that a conductive self-assembling peptide hydrogel could be engineered to be delivered in a minimally invasive manner and act as a scaffold for spinal cord repair. By combining the highly tunable and commonly used hydrogelator, 9-fluorenylmethoxycarbonyl-diphenylalanine (Fmoc-FF), with the conductive polymer, poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS), we demonstrate a preparative method for conductive self-assembled peptide hydrogels for spinal cord repair.

Development of Theranostic Gold Nanoparticle Encapsulated Systems: Aqueous Synthesis and Characterization of Gadolinium-Tethered Nanogels

Cliona Ní Chochlain, Nazila Kamaly, Alkystis Phinikaridou and James D. E. T. Wilton-Ely

Cancer is a leading global cause of death with increasing cases and associated deaths annually. Therefore, enhanced diagnostics and treatments are essential for improved patient outcomes.

Magnetic resonance imaging (MRI) is an invaluable diagnostic tool due to its high spatial resolution and absence of ionizing radiation. The development of MRI contrast agents based on trivalent gadolinium (Gd+3) has led to improved visualisation of internal structures and abnormalities such as tumours. However, clinically used contrast agents lack sensitivity. This stems from the low inherent relaxivity of the contrast agents. Nanomaterials have been found to dramatically improve relaxivity through the restricted motion achieved when tethering the Gd units to their surface.1 Nanogels are widely regarded as the ideal platform for payload delivery of therapeutic molecules, imaging agents or a combination of the two.2 These nanomaterials consist of flexible polymeric networks with high water retention while maintaining their structure and display a lack of toxicity.

This research focuses on the preparation of theranostic Gd functionalised nanogels with encapsulated gold nanostructures for combined MRI and photothermal therapy applications. The relaxivity of these nanogels was determined to be substantially greater than the clinical standard, DotaremTM. However, while the presence of gold nanomaterials within the nanogels is encouraging, further work is required to achieve the desired morphology suitable for optimal photothermal efficiency.3 This has motivated the exploration of an alternative novel nanogel synthesis method.4

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Co-assembled peptide emulgels: Promising adjuvanted vehicles for nasal delivery of influenza subunit vaccine

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Peptide emulgels have attracted much interest in the field of drug delivery in the recent years, due to their unique properties being biocompatible and biodegradable products [1]. They also have tunable physicochemical and mechanical properties that qualify them as soft shear thinning materials able to be loaded with different types of pharmaceuticals, that can be utilized as sprayable drug delivery vehicles [2,3].

Thanks to the vaccines' developers, huge efforts were achieved to control seasonal influenza infection and contain catastrophic impacts of pandemics [4]. Influenza subunit vaccines consist of part of the virus that has the antigenic epitopes necessary to induce an immune response when administrated. They are considered safe compared to whole viral particle vaccines, but mostly are required to be co-administrated with adjuvant to boost their immune response. For instance, squalene has been used as adjuvant oil for the subunit Flu vaccine (MF59®, by Novartis) [5]. Although the nasal route of administration provides great advantages in terms of vaccine delivery, weak vaccine effectiveness is a challenge, as a result of the short residence time and mucociliary clearance in the nasal cavity; being vulnerable to be swallowed or deeply inhaled to the lung [6,7].

Here, we are introducing the development of peptide-based emulgels as mucoadhesive candidates for nasal delivery of influenza subunit vaccines. Counter charge ionic co-complementary peptides were mixed at physiological pH 7.4 forming nanofibres that have the ability to emulsify squalene oil, owing to their surface amphiphilic properties. We show formation of adjuvanted squalene emulgel with critical gelation concentration of 10 mg/mL of total peptide concentration. β-sheet structures were found to be predominant after the assembly, confirmed by ATR-FTIR and Thioflavin T fluorescence assay. SEM revealed the formation of interconnected network of nanofibres together with the formation of microspheres within the network, thanks to oil emulsification. In addition, these emulgels showed viscoelastic behaviour where their stiffness increases as a function of total peptide concentration and they have Gel-Sol-Gel thixotropic characteristic which have been investigated by rheological and sprayability studies.

These findings make the co-assembling peptide emulgels promising candidates that can be used as vehicles for the nasal delivery of influenza subunit vaccine.

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Biomimetic 3D In Vitro Models of Osteosarcoma: A Novel Hydrogel-Scaffold System for Enhanced Therapeutic Research

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Osteosarcoma (OS) is the most common malignant bone tumor affecting children, adolescents, and young adults. While standard treatments, including surgery and chemotherapy, have improved the 5-year survival rate to 66%, therapy resistance, recurrence, and adverse side effects remain major challenges. This underscores the need for innovative treatments. OS malignancy is strongly influenced by its complex tumor microenvironment (TME), which includes a small population of Cancer Stem Cells (CSCs) with self-renewing and pluripotent capabilities. The PREDICTOS project addresses this by developing 3D in vitro and in silico models that mimic the OS environment for enhanced therapeutic research.

This study presents a novel 3D *in vitro* model using enriched CSCs embedded in a hydrogel matrix as core within a bone-mimicking scaffold, aiming to replicate the CSCs niche in OS. The hydrogel is composed of 1% gellan gum (GG) and 0.3% hyaluronic acid (HA)—a polysaccharide derived from bacteria and a major extracellular matrix component, respectively. GG was solubilized at 2% (w/v) in water at 70°C, while HA was prepared at 3% in water at room temperature. CSCs were enriched over 10 days of induction by a well-established Sarcospheres-Forming Culture and embedded in the hydrogel ($3.2x10^5$ cells/mL). Then, the hydrogel was extruded through an 18G needle, with gelation initiated by cation solutions like PBS or cell culture media within minutes.

Preliminary evaluations of CSCs viability and morphology in the hydrogel up to 12 days (via PrestoBlue and live/dead assays) confirmed the cytocompatibility of the hydrogel system. Additionally, injecting the hydrogel into a bone-like scaffold demonstrated the feasibility of this model. These promising results suggest that this hydrogel-scaffold system could support the development of complex models for studying OS tumor behavior and for drug screening applications.

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Enhancing excellence for the development of advanced predictive and therapeutic models for osteosarcoma

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Introduction

Osteosarcoma (OS) is the primary malignant bone tumour that mostly affects children, adolescents, and young adults. Surgery and chemotherapy, as standard treatments used, improved 5-year overall survival in 66% of cases. Some concerns remain related to the resistance to standard therapies, the recurrence of the tumour and the adverse side effects on the patients, highlighting the need to develop novel treatment strategies.
The PREDICTOS project aims to address this need by exploiting biomimicry strategies to develop and investigate the best-in-class 3D in vitro and in silico models that mimic OS environment, with the main purpose of studying therapeutic mechanisms. Nevertheless, as a proof of concept of an innovative therapy based on magnetic materials and magnetic stimulation, to development of improved treatment therapies will be also investigated.

Results and Discussion

The present work highlights the development of a 3D in vitro predictive model composed of magnetic bone-like scaffolds and osteosarcoma cells. Collagen was mineralised with multiple-ions-doped hydroxyapatite by assessing to biomineralisation process and then engineered in scaffolds using the freeze-drying process.
The biological performance of the proposed scaffolds was evaluated in vitro as a potential Extracellular Matrix (ECM) for the development of predictive OS systems. The scaffolds were seeded in vitro with different osteosarcoma cells (MG63, SAOS-2 and U2 cell lines) and the cell-ECM interactions and the cellular behaviour were investigated in terms of cell proliferation and viability, cell morphology and colonization, together with the specific immunolocalization of cellular adhesion-related markers.

Conclusions

The promising preliminary results obtained in terms of cytocompatibility and promotion of cellular colonisation by the proposed systems will drive the synthesis of more complex models using other revolutionary technologies (such as 3D printing), biomaterials or physico-chemical signals.

Acknowledgements

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New Approaches in the Production of Textured, Cultured Meat

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Objectives: To address the need for scaffolds in lab-cultured meat production to achieve high-quality meat at large scale and low cost we have investigated the design, characterization, and testing of hydrogel materials as scaffolds to enhance meat culture in bioreactors. We employed 3D bioprinting to assess the potential of these printed scaffolds in supporting the growth of structured, cultured meat. Our focus is on optimising a variety of hydrogel materials for the cultivation of meat cells, specifically mouse muscle cells. By integrating slow-releasing growth factors such as TGF-b1 and IGF-1 into the hydrogels, we aim to identify optimal formulations that promote cell proliferation, support material growth, and remain cost-effective.

Methods: We conducted physical characterization of various peptide hydrogels [1][2] to evaluate their suitability for bioreactor culture. This included assessing the mechanical properties and structural integrity of the hydrogels. 3D cell cultures of mouse muscle cells were established [3], alongside the design of microcarriers to support cell growth. The biocompatibility of these hydrogels was evaluated by examining cell proliferation using biological assays, and further analysed through Raman spectroscopy and confocal microscopy to assess cellular interactions and material performance.

Results: Three animal-free hydrogels were evaluated for their initial biocompatibility with C2C12-GFP cells. Under standard culture conditions, the cells were allowed to differentiate into myotubes, and differentiation was assessed using Raman spectroscopy and confocal microscopy to determine which hydrogel was most favourable for cell expansion and development. Microcarrier cultures of the C2C12-GFP cells were investigated, revealing significant differences in cell activity following the introduction of differentiation media, supporting their use in bioreactor culture. Additionally, bioprinting cultured meat was demonstrated to be feasible using self-assembling peptide hydrogels, highlighting their potential for structuring lab-grown meat.

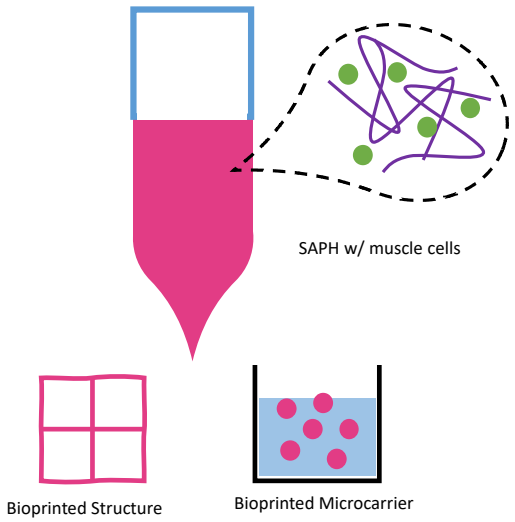

Figure 1: Bioprinting lab cultured meat

Conclusions: We have demonstrated that lab-cultured meat can be successfully grown in animal-free, self-assembling peptide hydrogels. The versatility of these hydrogels as scaffolds highlights their potential for future applications in cultured meat production.

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A thermo-responsive shape memory polymer for the delivery of an implantable hypertension sensor

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Introduction

Significant blood pressure fluctuations in patients can result in stroke and other cardiovascular events. For patients at high risk of high blood pressure, therefore, it can be valuable to continuously measure of blood pressure over time (1). Current methods of blood pressure measurement such as in a clinical setting or electronic monitors at home are subject to fluctuation or can only take single measurements (2,3). Here we report on the development of a shape memory polymer that can be used to fabricate a blood pressure sensor and will unfold following minimally invasive delivery into the body. Here we report on the development of a polycaprolactone (PCL)-polydimethylsiloxane (PDMS) shape memory polyurethane (SMPU) comparing its properties, at various PCL:PDMS ratios, to that of PDMS, which is non-shape memory but has previously been used to fabricate a blood pressure sensor.

Methods

The PCL-PDMS SMPU was synthesised by first dissolving both PCL-diol and PDMS-diol at mass ratios of 4.0:1.0, 3.5:1.5, 3.0:2.0 and 2.8:2.3 in 25mL anhydrous toluene at 60ºC. Once a homogeneous mixture was achieved 290µl of hexamethylene diisocyanate and 2µl of dibutyltin dilaurate were added to the mixture. The mixture was reacted under argon at 110ºC, then precipitated in n-hexane and filtered under reduced pressure to produce a solid product (4). FTIR and NMR were used to characterise the polymer structure, DSC was used to characterise the polymer's thermal properties. The mechanical properties have been tested by first solvent casting the SMP, using chloroform, into circular disk specimens that undergo cyclic compressive test where samples are compressed for 10,000 cycles at 20% strain and a frequency of 1Hz. Specimens were also cast into sheets which were used for shape memory testing.

Results and Discussion

It was clear that there was a distinct difference in the stiffness of these formulations with the higher concentration of PCL significantly stiffer and more brittle, with PCL:PDMS ratios of 4.0:1.0 and 3.5:1.5 failing within a few cycles. Whereas the formulations with a higher PDMS content were comparable to those exhibited by the pure PDMS. Spectra produced by both NMR and FTIR confirmed successful polymerisation. DSC showed the polymer transition temperature to be between 45-48ºC. Shape memory testing showed that the polymer exhibited a shape memory transition.

Conclusion

Here we report that a PCL:PDMS shape memory polymer can be manufactured to exhibit similar mechanical properties to PDMS while exhibiting the benefits of shape memory properties. Further work is required to reduce the transition temperature to allow shape memory actuation at physiological temperature.

Acknowledgments

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The Development of High Strength Vascular Adhesives

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Abstract

Trauma remains a prominent and unavoidable cause of death worldwide. Although central nervous system (CNS) injury is the greatest mortality contributor here, death is often unavoidable in these cases. Conversely, bleeding to death (exsanguination) – the 2nd leading cause – is treatable[1]. Despite this haemorrhage outcomes have not significantly improved over the years[2].

Treating severe haemorrhage is a time-sensitive and multidisciplinary situation, involving numerous professionals and interventions, from the time of injury until patient stabilization is reached. This employs a myriad of techniques and technologies, including gauzes, tourniquets, medical imaging, suturing and stapling. However, these interventions are often insufficient, necessitating the need for rapid, simple methods of achieving haemostasis in a patient.

In recent years significant progress has been made in the field of tissue adhesives (bioadhesives). These now present many medical applications and a range of different formulations and functionalities. Even so, regarding vasculature, they are currently only approved as a surgical adjunct, thus not removing the requirement for complex and time-consuming procedures. This lack of progress largely stems from the associated complexity and extra stipulations required for tailoring tissue adhesives towards vasculature. This includes synthesis of materials with sufficient adhesive/cohesive strength to withstand blood pressure – largely stemming from chemical interactions of components –, mechanical properties in line with native tissue, and suitable gelation kinetics and mechanisms. In addition, the adhesive must present maximum biocompatibility, degradation in line with vessel remodeling and – ideally – self-healing capabilities.

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Quantifying the Effect of Cell Layers on Nanoparticle Diffusion in Experimental Models

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Introduction

Quantifying the effect of cell layers on nanoparticle behaviour can contribute to an understanding of the forces and mechanisms that govern nano-entity dynamics in biological environments, and consequently aid in the design and optimization of therapeutic nanoparticles. A highly accessible label-free microscopy technique is described that has the potential to quantify and characterise the influence of cell presence and interaction with the local environment on the dynamics of nanoparticles in a solution. Fluorescence microscopy techniques are one of the most widespread tools to monitor and track biological interactions at the nano scale; however, among other issues, the influence of fluorescent tags on diffusion and biological activity is still unclear. Experimental regimes that can characterise the influence of changes in protein concentrations in localized environments and protein corona formation on nanoparticle dynamics are needed to further our understanding of nano entity transport in complex biological environments. The aim of this study is to develop a model for the diffusion of nano-entities though biological media in the presence of a cell monolayer with a view to developing a technology platform for *in-vitro* test systems to monitor how the presence of a specified cell monolayer affects the zonal diffusion of nanoparticles as the nanoparticle approaches the cell monolayer

Method

Using a standard inverted optical microscope adjusted to produce near-coherent light to generate optical signatures of nanoparticles, or caustics, as described by Patterson and Whelan [1], positively- and negatively-charged gold nanoparticles as small as 50nm in diameter have been visualized and tracked above a cell layer. 100nm diameter gold nanoparticles were tracked diffusing at different heights above a human mesenchymal stem cell monolayer over time. The influence of nanoparticle charge, concentration and size on their dynamics in biological environments was also investigated.

Results and Discussion

Analysis of the values of the diffusion coefficients of the particles and the size of the convex hull enveloping their motion has shown that the local extracellular microenvironment of nanoparticles influences their diffusion. Significant changes in nanoparticle diffusion rate have been observed one hour after exposure to a cell layer and factors such as the presence of serum proteins, nanoparticle surface charge and cellular activity could potentially influence nanoparticle dynamics due to changes in nanoparticle physical properties or local environmental factors.

Conclusions

Experimental regimes to obtain quantitative information on the factors influencing nanoparticle diffusion in biologically relevant environments have been developed and optimised. The results may alter the conventional picture of the effect of nanoparticle size on nanoparticle diffusion and highlights the importance of considering protein interactions on nanoparticle dynamics.

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Advances Towards Industrial-Scale Manufacturing of Eco-Friendly Nano-Pharmaceuticals

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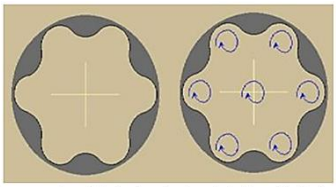
Nanotechnology has effectively pervaded the field of medical science, particularly in drug delivery. Notwithstanding the significant progress made in the domain of nanomedicines at the laboratory level, a restricted proportion of nanotechnology-derived pharmaceuticals is accessible in the global market. This is due to the frequent utilization of hazardous chemicals in traditional nanoparticle synthesis techniques. This study aimed to explore innovative, easily scalable, and environmentally benign methods for the preparation of drug-loaded nanoparticles at a pilot scale, avoiding the use of toxic organic solvents. Furthermore, to locally engineer, fabricate, and enhance prototype pilot-scale apparatus for nanoparticle synthesis to facilitate the transition from laboratory to industrial scales. Carbamazepine-loaded transfersomes[1] (CZTs), a form of lipid-based nanoparticles, were synthesized on a small scale utilizing a modified scalable heating method that eliminates high-energy processes and the use of organic solvents (green synthesis)[2] in a custom-designed vessel that resembles Mozafari's beaker, featuring baffles that create multiple turbulences conducive to the formation of nanosized particles.[3] A three-factor, three-level Box-Behnken design was utilized to optimize process and formulation variables. Additionally, the optimized formula was synthesized on both a small scale and a pilot scale using the modified heating method. The small-scale beaker, simulating Mozafari's 50 milliliters capacity, was successfully scaled up to a pilot-scale tank with a capacity of 13 liters. Consistent outcomes were noted for the optimized formulation on both small and pilot scales, exhibiting a mean size of 323.12±0.3 nm and 341.5±1.4 nm, respectively, a high entrapment efficiency (EE) of 76.12±1.1% and 75.01±0.5%, respectively, and a sustained release profile. The study concluded that the optimized formulation of CZTs was successfully produced on both small and pilot scales utilizing a simple scalable, eco-friendly, modified heating method. The Box–Behnken surface analysis demonstrated efficacy in optimizing the CZTs formulations. The CZTs produced at both scales exhibited similar results, demonstrating uniform nanoscale dimensions, high encapsulation efficiency, and a sustained release profile.



A cross-section of the beaker, showing the baffles of the beaker and multiple turbulences created by magnetic stirring during the formulation of nanoparticles as introduced by Mozafari.

The six-baffled homemade glass vessel simulating Mozafari's glass bottle of 50 mL capacity.

The six-baffled pilot scale tank of 13 L capacity.

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Smart wound dressings with on-demand antimicrobial therapeutics

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INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune rheumatic disease that primarily causes fibrosis to the skin and internal organs (Denton and Khanna, 2017). Like most rheumatic pathologies, SSc is a chronic disease, requiring the majority of patients to undertake adverse treatments that have undesired side effects (Mura et al., 2012). One of the main issues arising from SSc is the reoccurrence of refractory digital ulcers (DUs) causing poor quality of life (QoL) and vast amounts of pain to patients (Hughes and Herrick, 2016). The aim of the study is to design a new wound dressing for DU management enabling on-demand antimicrobial therapeutics to promote an improved QoL to the patient. To realise this, the wound dressing will be designed to enable antimicrobial therapeutics via light irradiation, following the principles of Photodynamic Therapy (PDT).

MATERIALS AND METHODS

Collagen type I sourced from rat tail (CRT) was covalently functionalised with 4-vinylbenzyl chloride (4VBC), as previously reported (Tronci et al., 2013). The degree of functionalisation was determined by 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay. 4VBC-functionalised CRT (CRT-4VBC) was magnetically stirred into a 17.4mM AcoH solution supplemented with Irgacure 2959 (I2959) at room temperature for 24 hours. The photoactive solution was then cast into a 24-well plate and UV irradiated at 365 nm (Chromato-Vue C-71, Analytik Jena, Upland, CA, USA) on each side for 15 minutes. Fully cured samples were dried in aqueous solutions of increasing ethanol concentration. UV-cured collagen hydrogels were initially characterised with respect to their compression properties, gel content and swelling ratio. The compressive modulus was calculated by linear fitting of the stress-strain curve obtained from the Bose Endura tec ELF 3200. Rose Bengal (RB) and Toluidine Blue (TB) were selected as photosensitisers given their proven antimicrobial effects A calibration curve was generated for both photosensitisers via UV-VIS spectroscopy, prior to assessing the release profiles. Samples were drop cast using the method as previously outlined (Brooker and Tronci, 2023) with an aqueous solution of RB or TB in distilled water (DI), prior to air 24-hour air drying. The resulting samples were then incubated in DI and a sample of supernatant was removed at specific time points. The absorbance was measured at 562 nm and 630nm, to characterise the release profile of RB and TB, respectively. The loading efficiency of both photosensitisers was calculated by weight measurements.

The compressive Youngs modulus was calculated by linear fitting of the stress-strain curve obtained from the Bose Endura tec ELF 3200. Samples were punched with a 10mm punch to keep sample dimensions uniform due to the anisotropic behaviour of the hydrogel.

RESULTS

A mean compressive modulus (n=3) of ~120 kPa was measured from the stress-strain curves at 10-15% of ultimate compressive strength with a tensile modulus of 1.7±0.2kPa. A loading efficiency of ~100 wt.% was measured from the drop cast samples of both RB and TB compositions. RB was observed to release 5±6 wt.% after 24 hours. TB was observed to release 104±11wt.% after 24 hours.

DISCUSSION

Standardisation of compressive testing methods has been developed to ensure uniform sample dimensions and testing reproducibility. A new tensile testing methodology was also developed to gain further insight on the mechanical behavior of these UV-cured collagen hydrogels . Successful drop casting of both RB and TB allowed for their respected release profiles to be established. Low release profiles from RB are promising in inducing antimicrobial therapeutics while retaining the photosensitiser within the gel. The study determined that the use of TB was not suitable due to its complete release within ~4 hours. Further studies are planned for the systematic assessment of RB release from, and mechanical properties of, the UV-cured CRT hydrogels, alongside testing *in vitro* to assess the photodynamic effects on DU bacteria and the tolerability of mammalian cells.

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Engineering bio/material inks for bioprinting of multicellular and mechanically tuneable hydrogels

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Introduction

The extracellular matrix (ECM) is a three-dimensional (3D) network that provides mechanical and biochemical support to cells, often responsible for instructing their phenotypes. It is known that ECM variations in viscoelasticity, density, composition, and structure are highly involved in biological mechanisms (e.g., disease development, progression), but the triggers of this often remain unknown. Such scarce knowledge stems from the limited availability of appropriate 3D human-mimicking models able to recreate distinctive ECM features in vitro. As advances in 3D in vitro scaffold development continue, the ability to fabricate more physiological-like multicellular structures that mimic physical, biological, and structural features of the ECM becomes pivotal. This work reports on the development of alginate-based bio/material inks with tuneable mechanical properties, capable of reproducing healthy and diseased tissue-specific ECMs and supporting cells ingrowth.

Methods

Composite biomaterial inks of pristine alginate, oxidised alginate (OA), gelatin, and polyethylene glycol (PEG) were formulated to form hydrogels using calcium chloride as a crosslinker. Tissue-specific hydrogels capable of mimicking the mechanical properties of soft human tissue were obtained (elastic modulus between 1-15 kPa). Specifically, by varying the degree of oxidation of OA, molecular weight of PEG, the concentration of ionic crosslinkers, and the time for the Schiff base reaction between OA and gelatin, it was possible to map a range of target mechanical properties independently of the biochemical composition. Obtained hydrogels were tailored to different biological systems by decorating the PEG with tissue-specific peptides via Michael addition. ECM-mimicking hydrogels were mechanically tested using rheology and compression, and viscoelastic properties derived through mathematical models.

Results

The concentration of ionic crosslinker used was found proportional to the elasticity of the hydrogels (1-15 kPa). The Schiff base reaction time between OA and gelatin impacts the elasticity and density of hydrogels. PEG MW (i.e., OA side-chains) was found proportional to hydrogel viscoelasticity, measured by comparing the strain energy released immediately following the removal of an applied force. Between 0.01% and 0.5% strain energy is released within the first 0.5s of force removal in either shear or compression, which is in line with the values reported from human tissues. The lower the energy released, the more viscous the material.

Conclusions

Results show a highly versatile biomaterial ink library able to form hydrogels mimicking a wide range of tissue-specific environments for regenerative medicine and tissue engineering applications. In specific, these hydrogels offer a new technology to help in understanding on the role of substrate viscoelasticity in cellular behaviour and disease progression, with further use for the development of organ-on-chip technologies. The composition and fabrication of the hydrogel can be altered to tune the biological and mechanical properties of the resulting substrate. The hydrogels produced can be used to easily create biocompatible, 3D in vitro models to study disease states and cellular behaviour, and to investigate the effect of substrate mechanical properties on cellular adhesion, migration, proliferation, and differentiation.

Synthesis of antimicrobial polymeric ionenes and their evaluation in Galleria mellonella *in vivo* model

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Polyionenes are a class of ion-containing polymers characterised by the presence of quaternary nitrogen atoms incorporated directly into the main polymer chain, distinguishing them from other polymers where ionic sites are typically pendant [1]. This unique structural feature endows polyionenes with inherent cationic charge density along their backbone, which contributes to their interaction with negatively charged microbial membranes. Such interactions can disrupt membrane integrity, leading to antimicrobial effects.

In this study, various types of polyionenes were synthesized through the Menshutkin reaction of N,N,N′,N′-tetramethylethylenediamine with either bis(2-chloroethyl)amine hydrochloride or 1,2-dibromoethane, as well as by the reaction of linear polyethyleneimine with bromoethane.The structures and properties of the synthesised polymers were analysed using ^{1}H NMR and FTIR spectroscopy, thermogravimetric analysis, and dynamic vapor sorption measurements. All the synthesised polymers were fully soluble in water and demonstrated a strong affinity for it.

The antimicrobial properties of these polymers were assessed using an *in vivo Galleria mellonella* (wax moth larvae) model. Larvae were infected with *Staphylococcus aureus* via injection and incubated at 37 °C. Untreated larvae developed severe infections, resulting in death within 24 hours. In contrast, infected larvae treated with antimicrobial polyionenes—administered 1-hour post-infection—exhibited reduced infection levels and improved survival rates. These findings demonstrate that certain polyionenes effectively mitigate infection and prevent mortality in this model.

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Vaterite $CaCO_3$ Crystals as a Versatile Platform for Drug Delivery into Cells

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Modern drug delivery systems (DDS), including liposomes, polymeric particles, and lipid-based carriers, have advanced medicine by enabling controlled release, enhanced bioavailability and targeted therapy. Among these systems, calcium carbonate ($CaCO_3$) emerges as a strong contender, offering not only the benefits of DDS but also unique advantages such as biocompatibility, biodegradability, cost-effectiveness, and pH-responsive release.[1]

This study explores vaterite $CaCO_3$ crystals as vectors for intracellular drug delivery, leveraging their unique porous structure and tunable properties to enable efficient drug encapsulation and release. Advanced vaterite-based delivery vectors containing bioactives of various nature (paracetamol, cannabidiol, dextrans) were synthesized via co-synthesis, producing particles with diameters ranging from 2 to 30 µm. Particle sizes and distribution were controlled through specific reaction conditions such as stirring speed and time for the co-synthesis reaction. As for delivery of $CaCO_3$ crystals into the cells, in vitro studies revealed efficient internalisation of crystals by adipocytes, with smaller particles showing enhanced uptake and significantly higher internalization compared to fibroblasts, suggesting tissue-specific delivery potential. The crystals demonstrated excellent biocompatibility, enabling controlled cargo release over hours without impairing cellular function.

$CaCO_3$ crystals, particularly vaterite, show strong potential as an effective and sustainable platform for controlled and targeted drug delivery. Their unique properties - such as biocompatibility/degradability and ability to encapsulate bioactives of various nature - make them a promising alternative to traditional DDS. This research highlights the versatility and potential of vaterite-based systems to revolutionize drug delivery, paving the way for broader therapeutic applications in inflammatory, metabolic and related disorders.

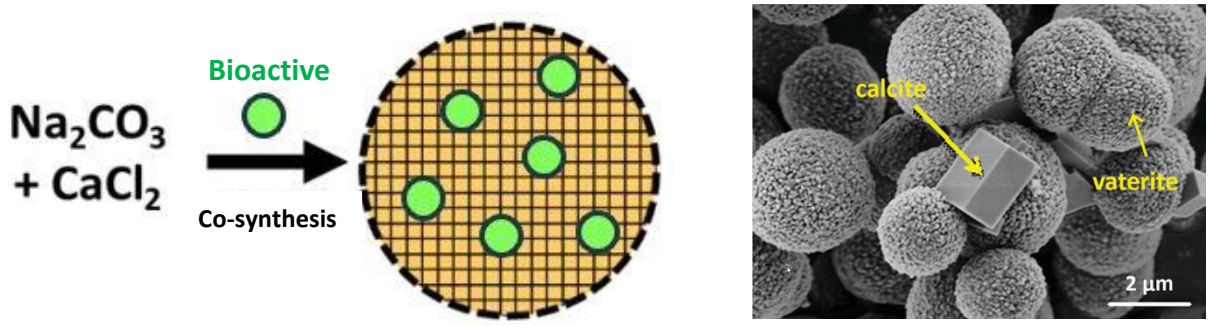

Figure 1. Left - schematic of co-synthesis of $CaCO_3$ crystals with bioactives. Right – scanning electron microscopy images of vaterite crystals, adopted from.[1]

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Cationic Polymers for Gene Delivery in Colorectal Cancer: Impact of Protonation Behavior and DNA Complexation

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Introduction: In 2022, almost 20 million new cancer cases were diagnosed globally[1]. Estimates indicate that about one in five people will develop cancer at some point in their lives[1]. Gene therapy, specifically RNA interference (RNAi), offers a promising approach to target and silence oncogene-related mRNA in various cancers. However, siRNA delivery faces challenges due to its hydrophilic nature and negative charge, limiting cell membrane penetration. The pursuit of developing safe and efficient non-viral gene delivery systems remains a complex challenge, necessitating continuous exploration and refinement of polymer-based strategies for enhanced biocompatibility and therapeutic efficacy. To address these challenges, we have developed amphiphilic cationic polymers (ACPs) with different cationic nature for delivering double stranded silencing RNA (dsiRNA) in CT26 colorectal cancer cells. The major focus of this investigation is understanding the influence of cationicity in delivering the dsiRNA to the mammalian cells.

Results: Our study (Figure 1) shows that diblock copolymers that contain primary amines (AEAm) exhibited gene silencing above an N/P ratio of 20. The same trend was observed for polymers containing quaternary amine (TMAEAm), however, due to the permanent cationic charge of these polymers across the pH range, a higher degree of silence was also observed. On the other hand, the study showed that secondary amine (DMAEAm)-containing polymers have no activity, even at the highest N/P ratio tested (N/P = 40). Notably, our quaternary amine-containing polymers demonstrated higher transfection efficiency than the commercially available Lipofectamine at N/P ratios above 30, with no associated toxicity.

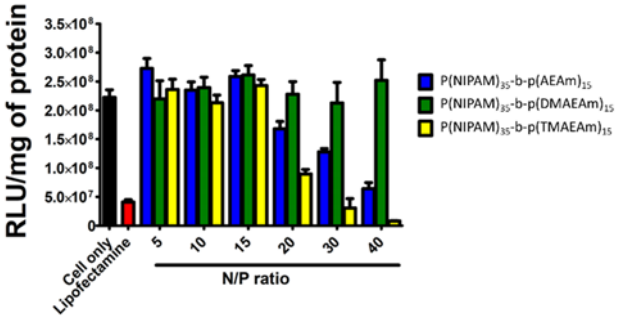

Figure 1. Luciferase gene silencing efficacy of ACPs in CT26-Luc2 cell line.

Conclusions: This study has shown that TMAEAm polymers hold potential for effective gene delivery in colorectal cancer, offering strong gene knockdown capability and favorable synthetic properties. Building on the promising results with TMAEAm polymers, our current experiments are focused on further optimizing the polymer properties. Specifically, we are investigating how the polymers' protonation behavior influences their ability to complex with pDNA at varying N/P ratios. Our work involves polyacrylamide and polymethacrylate polymers with different architectures (e.g., homopolymers, diblocks, triblocks) and amine types (primary and secondary) to enhance gene delivery potential under physiological conditions.

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RSC Biomaterials Chemistry Group 19th Annual Meeting

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LIQUID CRYSTAL FORMATION IN OLIGONUCLEOTIDE SOLUTIONS AND CHARACTERISATION OF THEIR MOLECULAR STRUCTURE

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Introduction: In recent years, regulatory agencies have markedly increased the approval rate of oligonucleotide therapeutics [1]. This class of therapeutics has demonstrated broad applicability across various medical domains, including ophthalmology, cardiovascular medicine, and neurology, utilising platforms such as siRNA, saRNA, and miRNA [2]. Nonetheless, the innovative nature of oligonucleotide therapeutics introduces unique challenges in development, notably due to limited understanding of their structural behaviour in solution. The viscosity of oligonucleotide-based formulations can exhibit unpredictable behaviour which poses potential obstacles for their manufacturing processes and patient administration. This study investigates the molecular interactions that drive liquid crystal formation, observed in certain nucleic acid sequences at high concentrations, and seeks to correlate these interactions with the resulting viscosity in oligonucleotide solutions. [3] Ultimately, we aim to identify the structural features that contribute to elevated viscosity levels in these systems.

Methodology: A solution containing a mixture of Poly(A) and Poly(U) in water and a solution of Poly(A) at 170 mg/mL are used as RNA-based oligonucleotides sequences. Crystal formation is confirmed through the observation of birefringence using a polarized microscope. The molecular structure of oligonucleotides in solutions is analysed via Infrared Spectroscopy (FTIR).

Results & Discussion: Birefringence is observed in the mixture but is absent in Poly(A) alone. The combined effects of hydrogen bonding and end-to-end π-π stacking of the bases promote the organization of chiral nematic liquid crystals [3]. FTIR spectroscopy provides insight into nucleobase pairing and the sugar puckering of the pentoses within this system, confirming in the mixture the formation of a predominantly double-stranded structure while also indicating that some uracil bases remain unpaired in solution. Additionally, the FTIR data reveals that the pentose sugars predominantly adopt a C3'-endo conformation, suggesting an A-form geometry (**Fig. 1**).

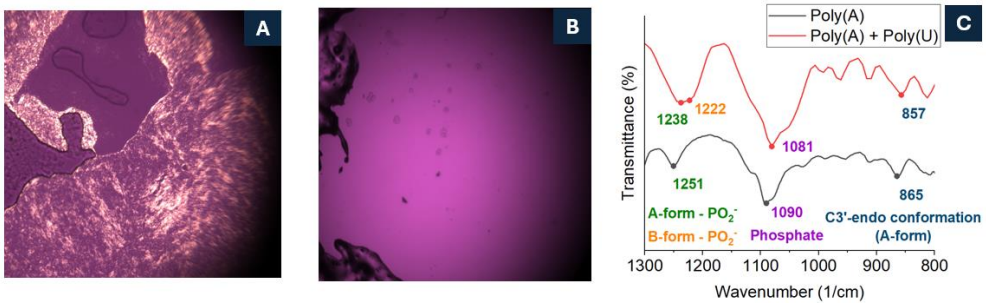

Fig 1. Polarised microscope images of 50:50 (mass-%) of Poly(A) and Poly(U) **(A)** and Poly(A) **(B)** in water at 170 mg/mL. **(C)** Stacked FTIR spectra of Poly(A) (black) and Poly(A)+Poly(U) (red) in the sugar/phosphate absorption area

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pH-Responsive Virus-shaped Mesoporous Silica Nanoparticles for Extra-Pulmonary Tuberculosis treatment

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Abstract: Utilizing nanoparticle drug delivery systems (DDSs) is an effective way to overcome hepatotoxicity and potential systemic toxicity caused by anti-tuberculosis (TB) drugs acting directly with the cells in the body, and enhance their therapeutic efficacy. In this work, biodegradable pH-responsive virus-shaped mesoporous silica nanoparticles (MSNs) capped by chitosan were synthesized to load the first-line anti-TB drug isoniazid as a DDS for treatment of extra-pulmonary tuberculosis (EPTB). The chitosan coating should improve the stability of anti-TB drugs in the blood circulation and allows the anti-TB drugs to act on mycobacterium tuberculosis (M.tb)-infected macrophages in the acidic environment of the M.tb bacteria. The virus-shaped MSNs could deliver drugs inside the bacteria, improving the therapeutic effect. This new DDS based on virus-shaped MSNs has great potential in EPTB treatment.

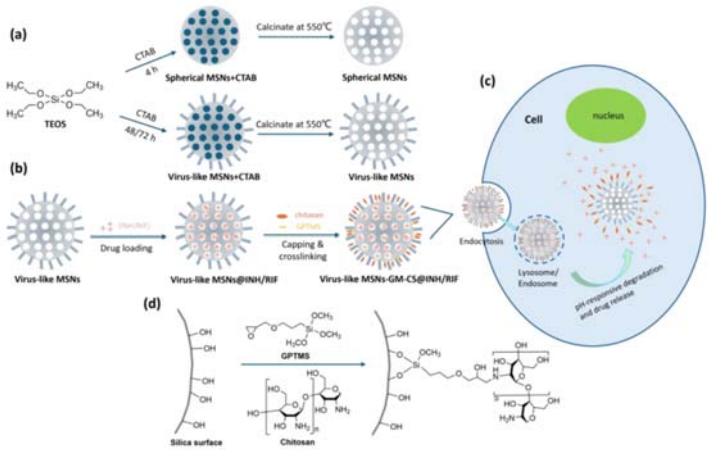

Figure 1. (a) The synthetic route of spherical MSNs and virus-shaped MSNs. (b) The synthetic route of virus-shaped MSNs-GM-CS@INH/RIF. (c) pH-responsive degradation and drug release of virus-shaped MSNs-GM-CS@INH/RIF inside the cells. (d) The chemical bonding process of chitosan, GPTMS, and silanol groups on the silica surface

Keywords: Virus-shaped mesoporous silica nanoparticles, pH-responsive, drug delivery, tuberculosis

Cell penetrating peptides for enhanced intranasal drug delivery

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Introduction

Effective drug delivery to the brain poses significant challenges, with many available methods being highly invasive. Cell-penetrating peptides (CPPs), such as cationic polyarginines, have emerged as promising enhancers for drug transport across the blood-brain barrier via intranasal administration. The length and structural characteristics of CPPs are thought to critically influence their drug delivery efficiency [1-3]. This study explores how the chain length of polyarginine (Rn) affects intracellular uptake, permeation mechanisms, and modulation of tight junctions, providing key insights into their application in intranasal drug delivery systems.

Results and Discussion

Our results demonstrate that polyarginine CPPs of varying lengths effectively permeate *in vitro* models relevant to intranasal barriers, with permeability strongly influenced by peptide length and structural attributes. Additionally, polyarginine was observed to modulate tight junction integrity, suggesting a mechanism for enhanced transport. These findings indicate that optimising polyarginine length could serve as a strategic approach to maximise intranasal permeation. Furthermore, cytotoxicity assessments confirmed that the tested CPPs are nontoxic to mammalian cells relevant to intranasal applications.

Conclusion

This study highlights the potential of polyarginine CPPs as permeation enhancers in intranasal formulations for brain delivery of target therapeutics, including anti-cancer drugs.

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Design of PVA hydrogels with self- assembled peptide for drug delivery applications

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Introduction: Polyvinyl alcohol (PVA) hydrogels have been widely used in medicine in recent years and have become ideal candidates for drug delivery systems due to their good biocompatibility, tunable mechanical properties and self-healing properties[1]. By embedding self-assembled peptides into PVA hydrogels, their biofunctionality and drug carrier capabilities can be further enhanced. The self-assembled peptides can form nanostructures that provide efficient encapsulation and controlled release of drugs and targeting through their interaction with the extracellular matrix[2, 3]. The composite hydrogel system not only has self-healing function to extend its service life, but also can achieve controlled release of drugs, reduce side effects and enhance therapeutic effects, which is particularly suitable for drug delivery applications such as tumors and chronic diseases.

Method: The composites were prepared by mixing PVA solution with self-assembled peptide solution and physical cross-linking was triggered using freeze-thawing method. Preparation of PVA solution requires heating and stirring in a water bath at 80-100 °C. After it was completely dissolved and cooled, the prepared self-assembled peptide solutions (1:1, 1:2, 1:4, 1:10) were added in different ratios and stirred well. Shear rheology was used to assess the mechanical properties and injectability of the new composites. The composites self- healing properties were also investigated. Differential scanning calorimetry and Fouriertransform infrared spectroscopy were used to observe changes in rystallinity and explore molecular interactions between peptide and PVA.

Results: The results show that the PVA hydrogel and self-assembled peptide composites are more stable and have higher storage modulus, and the addition of PVA does not destroy the shear thinning of the peptide, and the composites with a high proportion of PVA have some self-healing properties. For high molecular weight PVA at low concentration (<20 mg/ml) gels do not form instead viscous solutions are obtained. The addition of self-assembled peptide can make it gel. In addition, the self-assembling peptide conferred Ph sensitivity to the composite.

Conclusion: The composite hydrogel is significantly stronger and more stable mechanically than single PVA and self-assembled peptide hydrogels of the same concentration,and is expected to be a composite gel with self-healing properties. Future work will focus on finding suitable encapsulated drugs and therapeutic sites for this composite. The drug-carrying capacity and release mechanism of the composite will be further explored.

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key words: Peptide, PVA, Hydrogel, Drug delivery.

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RATIONAL POLYMER DESIGN FOR MICROARRAY TRANSDERMAL DELIVERY

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Introduction

Microneedle devices offer a painless method for delivering therapeutics, particularly biologics like peptides. These arrays can stabilize drugs and enable controlled release through the dissolution of the polymeric material forming the needles upon skin insertion. However, the interplay between polymer excipients and microneedle fabrication remains poorly understood, hindering rational design and broader adoption. This project aims to bridge this gap by evaluating polymers and fabrication parameters to correlate polymer properties with microneedle mechanics and drug delivery performance.

To accomplish this, acrylamide-based polymers with defined chain lengths and narrow dispersity have been synthesized for microneedle backing layers, which provide structural support and enhance skin penetration. These layers were tested using an in vitro skin model and compared with commercial polymers (PVA and CMC). Machine learning was employed to predict optimal microneedle compositions, streamlining design and reducing experimental workload.

Results and Discussion

Structurally diverse polyacrylamides, both homopolymers and copolymers, were synthesized by ultra-fast RAFT polymerization, offering precise control of molar mass, and creating polymers with narrow dispersity. Their chemical properties were characterized by ^{1}H-NMR, SEC, and DSC techniques. Microneedles, with differing backing layers, were fabricated using commercially available polymers alongside the synthesised ones. A parafilm *in vitro* skin model was used to insert the microneedles, using a texture analyser, and their insertion performance was measuring by viewing the punctures created under a microscope. Machine learning was then used to analyse the data and identify factors that influence microneedle insertion depth.

^{1}H NMR, SEC, and DSC data analysis confirm composition, chain length, low dispersity and glass transition temperature of polymers. The *in vitro* skin model data shows that differing backing layers confer a difference in microneedle insertion performance. Initial data visualisation showed that there were no linear trends between polymer properties and microneedle insertion performance. Machine learning was used to identify factors that influence microneedle insertion performance through non-linear relationships.

Conclusions

Chemical characterisation indicates that RAFT polymerisation offers a quick approach to producing acrylamide and acrylate-based polymers with well-defined chain lengths and narrow dispersity. DSC data reveals that the synthesised polymers adopt a hard/glassy state, justifying the need for plasticiser in polymer blends. Following insertion into an *in vitro* skin model, data analysis showed that polymer descriptors were strongly correlated with each other but showed no linear correlation with microneedle insertion performance. Machine learning was used to identify non-linear relationships that influenced microneedle insertion performance.

Multicomponent supramolecular hydrogels for nerve repair

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Introduction. Nerve repair poses a significant challenge in regenerative medicine field due to the intricate and time-sensitive nature of neural tissue regeneration.[1] Current treatment methods rely on using nerve autografts, allografts, or xenografts for larger gaps and suturing of the nerves for smaller gaps. Unfortunately, these approaches have drawbacks such as limited tissue availability, complex harvesting, scarring, insufficient nerve regeneration and neuron misguidance.[2] Hydrogel-based biomaterials are promising candidates for nerve regeneration as they present several advantages such as relatively simple and cheap manufacturing, injectability, biocompatibility and mechanical and chemical tunability.[2] Low molecular weight (LMW) hydrogels are composed of gelators that have a molecular mass of <1000 Da and exhibit the ability to self-assemble in response to application of a trigger such as pH, temperature, or solvent change. LMW hydrogelators are often composed of small building blocks such as peptides and present further advantages over regular hydrogels such as easy synthesis and functionalisation, and effective removal from the body through the renal system.[3] When developing hydrogels for nerve repair, material stiffness and conductivity are some of the most important properties to consider as both have been shown to control many neural cell processes influencing tissue regeneration such as cell migration, adherence, and proliferation.[4] In this project hydrogels formed by two supramolecular LMW gelators, naphthalene functionalized with diphenylalanine (2-NapFF) and perylene bisimide appended with leucine (PBI-L), were studied. 2-NapFF is a well-known self-assembling compound that can be used to form hydrogels exhibiting storage modulus (G′) of ~15 kPa,[5] whereas perylene bisimide (PBI) based gelators exhibit conductive properties and ability to self-assemble, especially when functionalised with amino acids at the imide position.[6] The aim of this study is to create mechanically tunable and conductive hydrogels using multicomponent hydrogel system composed of the two supramolecular gelators.

Results and Discussion. PBI-L hydrogels were successfully formed in PBS using the salt addition method. From the rheology results it was observed that pure PBI-L hydrogels exhibited G′ of ~1 kPa which is equivalent to some of the softest tissues in the body such as the brain.[7] In order to develop hydrogels for peripheral nerve repair, mechanical strength of the PBI-L hydrogels needs to be increased. To increase hydrogel stiffness, pre-gelled PBI-L and 2-NapFF solutions were mixed at different concentration ratios to create multicomponent composite hydrogels. From the rheology results it was shown that mechanical strength of the composite gels can be tuned by varying 2-NapFF ratio within the pre-gelled PBI-L/2-NapFF solution and hydrogels exhibiting G′ values of ~10 kPa can be formed using this system.

Conclusions. PBI-L based hydrogels offer potential for nerve repair due to their conductivity and self-assembly. However, pure PBI-L gels have been noted to be very soft limiting their utilisation for nerve repair. In this study, it was shown that incorporating supramolecular gelators such as 2-NapFF can be used to create PBI-L based hydrogels with tunable mechanical properties. However, further work is required to investigate biocompatibility and conductivity of these hydrogels. Therefore, future experiments will focus on studying the effect of 2-NapFF addition on hydrogel conductivity properties and evaluation of cell viability when cultured on the composite hydrogels.

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Exploiting Multicomponent Reactions to Synthesise Biologically Compatible Polycations for Non-viral Gene Delivery

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Multicomponent reactions (MCRs), which combine 3 or more components in a single, one-pot reaction, have revolutionised our ability to access complex products at high atom economy, high yield and in mild solvents whilst providing orthogonality with multiple functional groups.[1] They represent an ideal strategy to impart complex functionality to synthetic macromolecules, with the potential to encode previously inaccessible chemistries, and there is a growing body of literature on MCRs and multicomponent polymerisations (MCPs) in polymer chemistry.[2]

Among MCPs, Passerini-3 component polymerisation (P3CP) and Ugi 4-component polymerisation (U4CP) remain among the most popular, exhibiting many of the textbook benefits of MCRs.[3, 4] The materials they produce offer considerable promise for the development of novel biomaterials, as their inherent biodegradability[5] and the diversity in their functionality in many ways mimics that of natural polymers,

which are frequently used as biomaterials. The additional chemical versatility that P3CP and U4CP derived materials offer allows the tailoring of properties towards desired applications, offering the potential to replace some of the natural macromolecules currently used.

There is considerable interest in expanding the toolkit for synthesis of polycations, as polycationic biomaterials have been used to enhance drug delivery into cells; as carriers for nucleic acid therapeutics and vaccines;[6, 7] and a number of polycations show intrinsic antibacterial and antifungal properties.[8]

However, the Ugi 4CR converts primary amines to amides, and competition with this reaction renders the P3CP incompatible with primary amine groups.[9] Therefore, the direct preparation of polycations by MCPs remains relatively unexplored. For these polymerisations to be translated towards polycations, there is a need for the development of new monomer families.

In this work, we generated a set of modular diacid monomers containing tertiary amine cores. We subsequently utilised these monomers as substrates for both P3CP and U4CP to develop two diverse families of novel polycations. The functional diversity of the polymers was then further enhanced by post-modification via click-chemistries. Biological investigations found that a number of the Ugi polymers were potent delivery vectors for RNA, whilst many of the Passerini polymers exhibited an unusual, cell induced luminescence, similar to aggregation induced emission. This amounts to the first reported use of P3CP for the direct synthesis of polycations, and the first report of U4CP materials being successfully employed for gene delivery.

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Sustained growth factor delivery from bioactive PNIPAM-grafted-chitosan/heparin multilayers as a tool to promote cell behaviors

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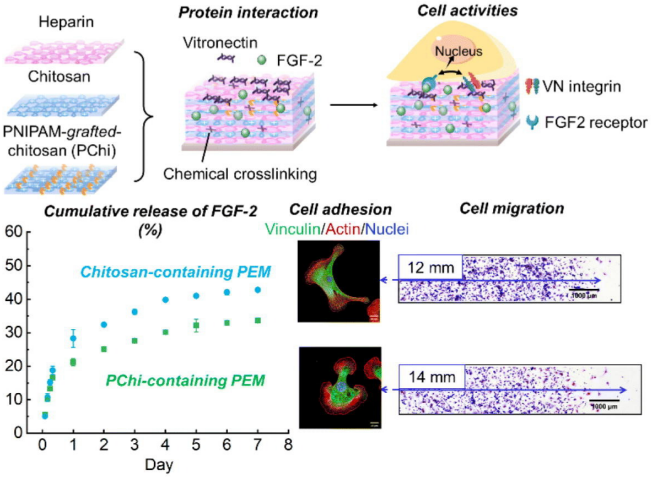
Abstract Delivery of growth factors (GFs) is challenging for regulation of cell proliferation and differentiation due to their rapid inactivation under physiological conditions in cell culture medium. Here, a bioactive polyelectrolyte multilayer (PEM) is engineered by the combination of thermoresponsive poly(N-isopropylacrylamide) (PNIPAM) and glycosaminoglycans to be used as reservoir for GF storage. PNIPAM-grafted-chitosan (PChi) with two degrees of substitution (DS) are synthesized, namely LMW* (DS 0.14) and HMW (DS 0.03), by grafting low (2 kDa) and high (10 kDa) molecular weight of PNIPAM on the backbone of chitosan (Chi) to be employed as polycations to form PEM with the polyanion heparin (Hep) at pH 4. Subsequently, PEMs are chemically crosslinked to improve their stability at physiological pH 7.4. Resulting surface and mechanical properties indicate that PEM containing HMW is responsive to temperature at 20 ◦C and 37 ◦C, while LMW is not. Particularly, HMW-containing PEM with Hep terminal layer shows not only better retention of the adhesive protein vitronectin but also sustained release of FGF-2 at 37 ◦C. With the synergistic activity of vitronectin and PEM-bound FGF-2, significant promotion on adhesion, proliferation, and migration of fibroblasts is achieved on HMW-containing PEM compared to Chi-based PEM and soluble FGF-2. Thus, PEM containing PNIPAM in combination with glycosaminoglycans like Hep represents a versatile approach to fabricate a GF delivery system for efficient cell culture, which can be potentially served as cell culture substrate for production of (stem) cells and bioactive wound dressing for tissue regeneration.



Figures 1 – Illustration of bioactive multilayer based on heparin and PNIPAM-grafted-chitosan (PChi) as a growth factor delivery system. PChi-containing PEM exhibits sustained FGF-2 release resulted in better fibroblast adhesion and migration.

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Reversibly adhesive thermo-responsive peptide hydrogels for localised drug delivery

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Introduction Over the past two decades hydrogels have seen a significant increase in research popularity for drug delivery as their highly tunable properties allow researchers to develop localised, slow and controlled drug release systems to reduce system toxicity and increase treatment efficacy.[1,2] Thermo-responsive hydrogels stand as the most widely researched environmentally responsive systems with particular attention given to poly(N-isopropylacrylamide) (pNIPAAM) for use in drug delivery because of its excellent biocompatibility.[1,3] However, current iterations of pNIPAAM systems suffer from poor biodegradability and therefore have limited use in biomedical systems. [3,4] We propose a temperature controlled reversibly adhesive peptide hydrogel based on the exposure of catechol groups as adhesive promoters. The hydrogels are designed to have excellent biocompatibility and biodegradability as they are constructed by the co-assembly of our previously reported peptide hydrogelator, the PhgEPhgK (Phg4) peptide and the 3,4-dihydroxyphenylalanine (DOPA)-Phg4, which are mixed with pNIPAM as the thermo-responsive component.[5] DOPA functionalisation is expected to enhance both mechanical properties and bio-adhesion of the hybrid hydrogels, making them more appealing for use in localised drug delivery applications.[6]

Methods Molecular self/co-assembly was investigated using ATR-FTIR. Mechanical properties were tested using oscillatory rheology, tensile and compression testing. Mesoscopic properties of the developed hydrogels were studied using electron microscopy techniques. Bio-adhesion was tested using lap shear tests.[8]

Results and discussion In this study we characterise hybrid hydrogels of Phg4:DOPA-Phg4 peptides in ratios 1:0, 0.75:0.25, 0.5:0.5, with/without pNIPAM. We begin by testing the stability of the hydrogels in the range pH 1 to 14. The swelling and shrinking kinetics of each of the hydrogels was tested over a range of temperatures (4, 15, 25, 37, 50 °C) where the swelling ratio of the hydrogel at 4 °C is taken as the equilibrium value after 24 hr incubation period. The swelling ratio defined as (Ws+Wp)/Wp where Ws is the the weight of absorbed water and Wp is the weight of the dried hydrogel.[7] Our data will help accurate determination of phase transition temperatures and further optimisation of the hybrid hydrogels designs to provide the required responsiveness under physiologically relevant environmental conditions. Bio-adhesive properties will be tested on artificial membranes covered with mucus and on porcine intestine tissue, as a function of DOPA content, to decide on the optimal hydrogel(s) for localised drug delivery in the colon.

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Fabrication of Antibacterial NO Releasing Cellulose Acetate Nanofibers for Wound Healing Applications.

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Introduction

Chronic wounds represent a major problem for the quality of life of patients and healthcare systems.[1] Infection is one potential reason the major reasons that wounds fail to heal or turn chronic.[1] Treating chronic wounds is problematic, given the rise in multidrug resistant bacteria and hence there is an urgent need to develop alternatives to antibiotics. Nitric oxide (NO) is a promising alternative to antibiotics because it has a multimechanistic way of killing bacteria and therefore there is a low chance of the bacteria developing resistance.[1] As NO is a gas, the delivery of NO to the site of the infection can be challenging. Electrospinning is a cost effective and easily scaleable manufacturing method of which can fabricated nanofibrous mats of high porosity and surface-to-volume, ideal for wound dressing materials and can be modified with NO releasing compounds.[2] In this study we report on the fabrication and characterisation of NO-doped cellulose acetate (CA) electrospun nanofiber mats and their antimicrobial efficacy against both gram negative and gram positive bacteria with no cytotoxicity observed and improved wound closure.

Materials and Methods

CA nanofibers containing a NO donor were produced by electrospinning. CA 15wt% was dissolved in a DMAC:acetone solvent system. The resultant solution of CA was electrospun at varying voltages to optimise the morphology of the nanofibers. The chemistry and the morphology of the of the nanofibers were assessed by Fourier-Transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Chemiluminescence was used to study the payload and release of NO. The antimicrobial efficacy of the fibres tested over 24 hours against Staphylococcus aureus and Pseudomonas aeruginosa in both planktonic and biofilm states. The cell viability and wound closure was investigated using HaCaT and WS1 cells.

Results and Discussion

The morphology of CA nanofibers were analysed by SEM and were between 378±300nm and 400±200nm. Tethering of the NO donors onto the CA nanofibers did not affect the morphology or the diameter. The chemical composition of the CA nanofibers were investigated before and after NO donor loading. Inclusion of the NO donor was confirmed by the presence of peaks in the FTIR representative of N-O stretching at 1550cm^{-1}. Chemiluminescence was used to analyse the release of NO from the fibres. An initial burst was seen, followed by sustained release over 24 hours. Antimicrobial testing demonstrated that NO-releasing nanofibers were able to effectively eradicate both planktonic and biofilms of *S aureus* and *P aeruginosa* after 24 hours. The nanofibers were found to be non cytotoxic to HaCaT keratinocytes and WS1 fibroblasts. Improved wound closure times were seen in both HaCaT and WS1 cells.

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