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NOVEL TREATMENT DISCOVERY

Autologous constructs for muscle engineering and repair

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Background

When volumetric muscle injuries overwhelm self-repair systems, endogenous tissue restoration often malfunctions. (1) Biofabrication as a promising pathway is urgently needed to effectively regenerate damaged skeletal muscles. (2) The objective of biofabricated constructs is to intricately replicate muscle tissue with cellular interactions that ultimately restore tissue physiology. Naturally derived polysaccharide hydrogels are a promising material vector due to their outstanding biocompatibility, biodegradability and tunability. (3) The constraint of adopting polysaccharide hydrogels is the absence of fibrous, mechanical and bioactive cues, which considerably confine their ability to emulate the extracellular matrix (ECM) of muscle tissue. (3)

Methods

We combine polysaccharide hydrogels with fluorenyl-9-methoxycarbonyl (Fmoc) self-assembling peptides (SAPs) that comprise ECM-protein-fibrous motifs to form uniquely adjustable mechanical, dynamic and physiological biocomposites. We investigate the mechanical and structural properties of these tailorable composited hydrogels via transmission electron microscopy (TEM), cryo-scanning electron microscopy (CryoSEM) and Small-Angle-Xray-Scattering (SAXS), the rheological characteristics using a parallel-plate rheometer, as well as printability test. We also examine the cytocompatibility of the designed bioinks in terms of cell viability, differentiation and migration behaviours.

Results

Our results show that the mechanics of biocomposites could be tuned by controlling the concentration and crosslinking of polysaccharides alone. We achieve a biofunctional stiffness range, bioactive micro- and nano-topography, mechanical and structural integrity and consistent nanofibrous assemblies by incorporated Fmoc-SAP polysaccharides networks.

Conclusion

We demonstrate that the formulation of Fmoc-SAPs with polysaccharide hydrogels has the biomimetic ability to meet the requirements of muscle tissue and ameliorates bio-printability outcomes.

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Baicalin enhanced neuroprotection and mitochondrial function in a human neuronal cell model

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Background

Baicalin is a flavone glycoside derived from flowering plants belonging to the *Scutellaria* genus. Previous studies have reported baicalin's anti-inflammatory and neuroprotective properties in rodent models, indicating the potential of baicalin in neuropsychiatric disorders where these processes are implicated. However, it is unknown whether these effects can be reproduced in a human neuronal cell model.

Methods

We treated NT2-N cells (human neuronal-like cell model) with three different doses of baicalin (0.1, 1 and 5mM) or vehicle control (DMSO) for 24 hours. To determine the transcriptional effects of baicalin on NT2-N cells, RNA extraction, genome-wide mRNA expression profiles and gene set enrichment analysis (GSEA) were utilised. We also performed neurite outgrowth assays and mitochondrial flux bioanalysis (Seahorse) in NT2-N cells treated with baicalin or vehicle control.

Results

We found in NT2-N cells that baicalin positively affected neurite outgrowth and transcriptionally up-regulated genes in the tricarboxylic acid cycle and the glycolysis pathway. Similarly, flux bioanalysis showed increased oxygen consumption rate in baicalin-treated NT2-N cells, an indicator of enhanced mitochondrial function.

Conclusion

Our findings have confirmed the mitochondria enhancing effects of baicalin in human neuronal-like cells, suggesting potential therapeutic application of baicalin in human neuropsychiatric disorders where these processes are operative.

Bio-inspired 3D materials for infectious wound healing

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Background

In everyday life, chronic infectious wounds are of immense concern due to long term patient discomfort and multiple hospital visits. Patients suffering from chronic wounds also tend to acquire secondary infection that makes the whole wound care management challenging. There is also evidence of increasing cases of chronic infectious wounds like Buruli ulcer in Australia, particularly in regions of Victoria [1,2]. Existing antibiotic therapies are challenging. Long term treatment regimens are required, necessitating multiple dosing which have concerns around developing resistance and side-effects that can affect vital organs. This opens a scope to strategize the optimal application of antibiotics via direct delivery of drug formulations to the site of insult. Here, we present a strategy for seaweed derived functionalised hydrogels with nanoparticles and anti-inflammatory components can provide a relevant 3D platform for smart release of antibiotics.

Methods

Seaweed derived alginate and fucoidan were used to prepare hydrogels infused with nanohydroxyapatite particles. The hydrogel was integrated with rifampicin and tested against for antibacterial effects. A classical wound scratch assay was performed to analyse the cell migration activities in the presence of the functionalised hydrogel.

Results

The functionalised hydrogel shows accelerated cell migration *in vitro* and antibacterial effects.

Conclusion

The bio-inspired functionalised hydrogels can be further explored for its wound closure ability and antibacterial effects against pathogenic bacteria including, MRSA and *Mycobacterium ulcerans* to provide sustainable solution against emerging chronic infectious wounds.

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Development of human pluripotent stem cell model of chronic fatigue syndrome for drug discovery

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Background

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating chronic disease characterized by severe fatigue exacerbated by activity. Its pathophysiology remains poorly understood, hindering effective treatments. Although classified as a neurological disorder, other clinical manifestations, such as impaired muscle function, suggest additional cellular involvement. To address this gap, we established an induced pluripotent stem cell (iPSC) biobank derived from ME/CFS patients and healthy individuals.

We hypothesize that ME/CFS is associated with an energy imbalance, particularly in skeletal muscle cells. Our project aims to: 1) differentiate iPSCs into functional skeletal muscle cells from ME/CFS patients and healthy controls (8 iPSC lines per group), 2) analyse metabolic and transcriptomic differences between these cells, and 3) identify potential drug candidates for ME/CFS treatment.

Methods

Patient recruitment is completed, and iPSCs are currently being generated from peripheral blood mononuclear cells using episomal vectors, and characterized (pluripotency, genotyping and karyotyping). The muscle cell differentiation protocol has been established, with differentiated cells exhibiting skeletal muscle cell markers (Myosin Heavy Chain, Titin and Myogenin), morphology (elongated rod shape), and spontaneous contractions. Our analysis will involve next-generation sequencing, metabolomics, bioinformatics, and bioassays for mitochondrial function, oxidative stress, and inflammation.

Conclusion

By using iPSC-derived skeletal muscle cells from ME/CFS participants, we will uncover novel insights into the cellular mechanisms underlying ME/CFS. Our findings may provide crucial information about energy imbalance in skeletal muscle cells, potentially leading to the identification of new therapeutic targets and repurposed drugs for ME/CFS treatment.

Designing gradient bioinks for the musculoskeletal interface.

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Abstract

A significant characteristic of the musculoskeletal system is the contiguous interface where the soft connective tissues such as cartilage, tendon and ligaments connect to the crystalline

bone [1]. This transition is a unique zone that ensures the stability of the joints by creating support around the joints [2]. These soft to hard tissue interfaces are prone to injury and are limited by their ability to self-repair. Thus, increasing the chances of risk to damage and decreasing the functionality. Restoring such gradient interfaces remains a major challenge in interfacial tissue engineering (ITE) because of their complex and dynamic nature [3].

A novel hybrid hyaluronic acid (HA) and peptide-based hydrogel approach was taken to construct a biomimetic tissue scaffold of defined porosity, with growth factors were incorporated to functionalise the material, we then used extrusion based 3D bioprinting technique to fabricate the physical gradients. In this method, multipotent stem cells were embedded into the hydrogel, and a controlled biochemical stimulus promoted cell differentiation. The mechanical properties of the tissue scaffold are optimised to match the natural tissue to withstand forces until the remodelling process ends as the joints are exposed to different mechanical stresses regularly.

The properties of the hydrogels were tested using differential scanning calorimetry (DSC), Rheology, Fourier transform infrared microscopy (FTIR), Cryo SEM and Atomic Force Microscopy (AFM). We demonstrate the synthesized novel bioink can bioprint tissue constructs with both physical and chemical gradients. The mechanical test results demonstrated that the synthesised hydrogels have certain structural properties that resemble the structure of the natural tissue. The biological evaluation of the hydrogels showed a gradient responsive control over cell fate, and measures of cell viability, cell attachment and cell proliferation. This research has recognised a potential novel bio-ink that can fabricate gradient tissue constructs that can mimic the host tissue while sustaining growth factor delivery to promote repair in tissue in the musculoskeletal interface.

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Engineering self-assembled peptide hydrogels for the osteochondral interface

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Background

Osteoarthritis (OA) is a highly prevalent disease, affecting upwards of 1 in 8 adults worldwide and is projected to increase by 60% in the next two decades.[1] The treatment methods for OA

are limited to removing or modifying existing cartilage. Fabricating, reproducing, and predicting cell to tissue formation are the key bottlenecks to translate to clinical applications.

Methods

Emerging tissue engineering approaches seek to address this challenge by utilising the cell's own capacity to produce an extracellular matrix (ECM) (material surrounding cell) under the guidance of specific exogenous cues, *i.e.,* both soluble factors and biophysical signals generated by cell-cell and cell-ECM interactions.[2,3] As such, we leverage the capacity of cells to self-organise and produce a tissue that has structural, morphological and functional properties comparable to the native tissue with functionalised peptide hydrogels.

Results

Here we show a new tissue engineering strategy to control the spatiotemporal behaviour of mouse bone stromal cells with self-assembled peptide hydrogels. As a result, these cells exhibit a specific gene expression profile that could map the gradient structure of the osteochondral interface.

Conclusion

The findings suggest that the progenitor cells can differentiate in response to predefined physical conditions of hydrogels to enable the fusion and remodelling of structurally organised tissues *in vitro*. Successful implementation of this research will enable us to realise the potential impact of high-resolution micro and nanoscale devices, which could provide new therapies for treating degenerative diseases including OA.

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Identifying a suitable technique to determine the status of differential DNA methylation

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Background

A multitude of techniques exists for detection and quantification of DNA methylation (DNAm). Primarily, there are three approaches: a chemical reaction using sodium bisulfite, cleavage of DNA with restriction enzymes (REs), and affinity capture of methylated DNA. In addition, there are few bisulfite, RE and antibody-free techniques (Khodadadi et al., 2021). These approaches can be combined with broadly practiced techniques such as Polymerase Chain Reaction (PCR), sequencing or microarrays to determine the status of DNAm (Laird, 2010). All

these techniques have benefits and limitations, however a universal technique that can be accessible by any basic
molecular biology laboratory requires development, careful optimisation and validation.

Methods

A literature search was conducted to identify the ideal technique for differential methylation analysis, using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Database used was PubMed with several query terms and exclusion criteria to filter the search.

Quantitative methylation specific PCR (qMS-PCR) and High-Resolution Melting (HRM) curve analysis was optimised and tested using HEK293, MDA-MB-231, Caco- 2, T98G and FHs 74 Int cell lines for 15 genes. The technique was validated using patient samples and microarray data from the Geelong Osteoporosis Study (GOS).

Results

The literature search revealed that quantitative methylation specific polymerase chain reaction (qMS-PCR) was the most economical and user-friendly technique that can be performed with available of equipment, reagents, with less time. and expertise required for data analysis. This technique was developed, optimised, and validated as an economical and efficient procedure to determine the status of DNAm.

Conclusion

This study identified that qMS-PCR with HRM was an effective technique to determine the status of differential DNAm.

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Investigating differential methylation of markers in Paediatric High-grade astrocytoma

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Background

Paediatric and adult astrocytoma are notably different. Understanding differentially methylated regions (DMRs) and their association with the aetiology of different diseases have improved prognosis of this lethal disease. We have tried to unravel the epigenetic modifications in Paediatric astrocytoma (pA) and signify the importance of studying these alterations which may have roles in predicting tumour progression.

Methods

A meta-analysis was done with 2513 studies. Methylation datasets were downloaded from NCBI and were analysed using an R- based differential methylation analysis tool, RnBeads. Differential methylation of CpG islands, CpG sites, promotors and genes were computed individually to correlate the effect of DMRs on DNA with respect to each other following which drug repurposing was done.

Results

Differential methylation analysis of CpG sites showed significant hyper-methylation at 211674 sites and significant hypo-methylation at 153807 sites. DMA of CpG islands showed significant hyper-methylation at 3980 sites and significant hypo-methylation at 16595 sites. DMA of promotors showed significant hyper-methylation at 13146 sites and significant hypo-methylation at 13690 sites. DMA of genes showed significant hyper-methylation at 6580 sites and significant hypo-methylation at 4187 sites (Samples having mean p value < 0.8, combined p value < 0.01 were considered hypermethylated and those having mean p value<0.2, combined p value < 0.01 were considered hypo methylated).

Conclusion

This study has identified significantly hyper and hypo methylated regions within the genome of patients with pA which are crucial in devising prognostic criteria and in studying the effects of epigenetic alterations in promoting tumour progression. We have also identified repurposed drugs based on these changes.

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Mitochondrial function is increased in lithium-treated neural progenitor cells derived from participants with bipolar disorder

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Background

Previous studies have shown mitochondrial dysfunction in people with bipolar disorder. This study investigated how mitochondrial function and energy production changes throughout the cell differentiation process from induced pluripotent stem cells (iPSCs) to neural progenitor cells (NPCs) and the effects of lithium on mitochondrial function.

Methods

Blood samples were collected from bipolar disorder (n=4) and healthy control participants (n=3). From these samples, peripheral blood mononuclear cells were isolated and reprogrammed using episomal vectors containing specific transcription factors to create iPSCs. These cells were then differentiated into NPCs. Both iPSCs and NPCs were then treated with lithium chloride (1mM) or vehicle as control. A mitochondrial bioenergetic profile was measured using a Seahorse XF24 Flux Analyser in the iPSCs and throughout differentiation into NPCs.

Results

Results highlighted the effects of time and treatment on the bioenergetic profile. iPSCs predominantly utilized oxidative phosphorylation for energy production. As iPSCs differentiated into NPCs, energy generation shifted towards the glycolytic pathway. There was no significant difference in mitochondrial function between groups. However, lithium treatment exhibited an effect by increasing mitochondrial function in both groups. Further exploratory analysis of NPCs indicated enhanced mitochondrial function post-lithium treatment in bipolar disorder cell lines but not in control-derived lines.

Conclusions

Preliminary data implies that mitochondrial function and bioenergetics are affected by lithium treatment, suggesting a potential therapeutical mechanism of action. Further analysis with a larger sample size is required for conclusive evidence. Nonetheless, initial findings look promising towards pinpointing beneficial effects of lithium on mitochondrial function.

Network-based drug repurposing for schizophrenia

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Background

Despite recent advances, drug discovery for schizophrenia remains challenging. Computational drug repurposing is a promising new methodology utilising expanding biomedical databases. Network analyses allow the comprehensive assessment of transcription factor regulatory effects via gene regulatory networks, reflecting transcription factor and target gene interactions by incorporating multiple lines of evidence.

Methods

We identified topological differences in the transcription factor-gene regulatory networks of schizophrenia cases versus unaffected controls using the PANDA algorithm. This incorporates binding motifs, protein interactions and gene co-expression data. The differential network was determined by comparing the strength of connections between the network associated with schizophrenia and the unaffected control network. The top positively differential transcription factors and negatively differential transcription factors were submitted to the CLUEreg tool of the GRAND database which utilises differential network signatures to find drugs that potentially target the disease's gene signature.

Results

Using a large RNA-seq dataset of 532 post-mortem brain samples from CommonMind, we built co-expression gene regulatory networks for schizophrenia cases and unaffected control subjects with 15,831 genes and 413 transcription factors overlapping. From drug repurposing results, 18 drugs were highlighted as top candidates for repurposing to treat schizophrenia.

Conclusion

Energy metabolism, immune response, cell adhesion, and thyroid hormone signalling are key pathways differentially regulated by transcription factors in schizophrenia cases compared to unaffected controls. Promising drug repurposing candidates, like rimonabant and kaempferol, show potential through these transcription factor-targeted pathways. Further preclinical and clinical investigations are needed to explore their mechanisms of action and efficacy in alleviating schizophrenia symptoms.

Phenotypic switch: Biomimetic hydrogels to direct the differentiation of bone marrow stromal cells via subtle changes in signalling environments

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Abstract

Bone regeneration is a well-orchestrated physiological process that involves the recruitment of bone stromal cells to the injury site, the sustained release of growth factors and support from the extracellular matrix.

Here, we will discuss how a tissue engineering approach has been geared towards developing 3D scaffold to mimic the natural bone regeneration *in vitro.* We demonstrate that a functionalised multifactorial hydrogel can effectively direct the differentiation of bone stromal cells without the need for growth factors.

The natural components of ECM are mimicked through use of a biomimetic triumvirate. Hyaluronic acid is used as an inert yet hydrated support, the protein components are

recapitulated with Self-assembled peptides containing signals representing laminin and fibronectin (IKVAV and RGD, respectively), and a method of controlling stiffness and mechanical properties via a covalent crosslinker (PEGDA). This yields a mechanically stable self-assembled hydrogel with nanostructure organization. These SAP-HA hydrogels are biocompatible and can effectively promotes bone stromal cells proliferation and differentiation.

We show, for the first time in such systems, dominant presentation of the RGD-motif triggers cells towards chondrogenic lineage with elevated expression of collagen II (COLIIAI), whereas promoting the IKVAV-motif induces osteogenic lineage with the expression of collagen 1 (COLIAI). Within hours of seeding, bone stromal cells attach to the scaffold and start forming elaborate spindle shaped self-organization. Phenotype rescue using dorsomorphin have shown scaffold induced differentiation phenotype mimics BMP2 pathway.

The biomimetic material has been tested on three cell lines can be potentially used as for a engineerered stem cell graft for improved muscloskeletal tissue regeneration.

Using a stem cell-derived model to repurpose drugs for bipolar disorder

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Background

Due to its episodic/cyclic nature, management of bipolar disorder (BD) is complex and reliant on medications from which many do not benefit. New treatment options for BD are urgently needed. We aim to identify off-patent drugs with known safety profiles that can rapidly translate to clinical practice to treat BD.

Methods

Sixteen research participants (8 BD and 8 matched (age, sex) healthy controls) were recruited for this study. Peripheral blood mononuclear cells isolated from blood samples were reprogrammed into induced pluripotent cells (iPSCs). iPSCs were differentiated into cortical networks (CNs), a mixture of neurons and astrocytes. CNs were harvested for RNA extraction and next-generation sequencing. The differentially expressed genes were used to run drug repurposing analyses completed using Connectivity Map (CMap, BROAD Institute) and LINCS2.

Results

The CMap analysis resulted in a list of compounds that could be repurposed for the treatment of bipolar disorder. Compounds with mechanisms of action associated with the

pathophysiology of BD, like ACE inhibitors, acetylcholinesterase inhibitors, and calcium channel modulators (and many more), have been suggested as possible repurposing alternatives. The fact that drugs like lithium chloride and quetiapine are also on the list further validate your approach, as these are commonly prescribed for BD.

Conclusions

The hypothesis-generating data from the CMap analysis corroborates our idea that by using the stem cell-derived *in vitro* model, we can bypass a fundamental roadblock in therapy development in psychiatry: the lack of singular molecular targets.