Department of Biomedical Engineering

The Chinese University of Hong Kong

BMEG 3430 Project Report

Biomaterial for Cartilage Tissue Repair and Regeneration

Group 3-5

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1 Introduction

This report aims to analyse the current medical needs and technologies for osteoarthritis (OA) and propose a novel solution to slow OA progression and promote regeneration.

2 Current Medical Needs

Osteoarthritis (OA) is the most common form of arthritis that affects 7% (~500 million) of the global population [1]. Ageing is strongly correlated to a decrease in cartilage elastic modulus [2], and therefore, one of the major risk factors of OA. Roughly 13% of women and 10% of men aged 60 or above suffer from symptomatic knee OA [3]. Notably, OA is not yet. reversible, and current non-surgical treatments can only reduce pain and alleviate the symptoms [4]. There is an urgent need for novel ideas to improve OA treatments.

Lubrication of cartilage within joints fosters tissue interaction and affects the mechanical properties of the tissue in joints. It acts as a boundary lubricant to provide nearly frictionless motion of joints [5, 6]. It is worth noting that the low concentration of lubricin can be observed in some OA cases, leading to decreased cartilage boundary lubricating function [7]. Lubricin is also able to protect articular chondrocytes against apoptosis [8]. Joint lubrication dysfunction is the main cause of OA, incurring loss of joint mechanical properties and tissue degradation. In cases of cartilage injury, structural damage occurs in the cartilage surface layer, leading to the loss of extracellular matrix (ECM) proteoglycans and disruption of collagen fibres.

Selecting suitable biomaterials for OA treatment involves balancing the physical properties to create materials that mimic natural cartilage's natural mechanical properties, including mechanical strength, stiffness, viscoelasticity and surface topography.

One must consider the cell-cell interaction and cell-ECM interaction for tissue regeneration and symptom alleviation, and the hydrogel must closely mimic the biological properties of cartilage, including immune response, osteointegration, and material exchange [9]. However, the existing hydrogels cannot perfectly align with the needs of cell regeneration for OA. In this regard, there is an urgent need for a novel technology to address these challenges.

3 Current Technologies

3.1 Hydrogels for Cartilage Repair

There are currently many hydrogel formulations targeted at cartilage repair. GelMA (gelatin methacrylate) is biodegradable, can undergo gelation with visible light, and has high, long-term viability of encapsulated human-bone-marrow-derived mesenchymal stem cells [10].

Physically cross-linked PVA (polyvinyl alcohol) hydrogel is biocompatible, biodegradable, chemically stable, and has high water content [11]. Chitosan-based hydrogel possesses drug-delivery capacities and antimicrobial properties [12].

However, these hydrogels suffer from poor mechanical properties, low solubility and integration, and lack of biofunctionality and bioactivity [11, 13, 14], making them inadequate for use in cartilage repair despite their advantages.

3.2 Doped Hydrogels for Cartilage Regeneration

Doping the hydrogel with drugs can provide therapeutic effects in addition to mechanical support, fostering cartilage regeneration. Multiple factors, including the drug's integration with hydrogel, side effects, and stability, should be considered when selecting a drug candidate.

Corticosteroid is an anti-inflammatory and pain relief drug. Due to its rapid action and short half-life, it is mainly used for short-term alleviation of osteoarthritis. However, it is worth noting that it may lead to osteoporosis and hyperglycaemia [15, 16].

Bone morphogenetic proteins promote the differentiation of mesenchymal stem cells into chondrocytes and stimulate the production of extracellular matrix components and bone growth; however, their side effects include ectopic bone formation and osteoclast activation, leading to joint dysfunction, osteolysis, bone subsidence [17], and potential tumour formation [18].

Common drug integration methods include stable chemical bonding via thiol-maleimide couplings [19] and crosslinking via Diels-Alder reactions [20].

4 Proposed Solution

4.1 Description

We propose a long-term solution by incorporating the lubricating properties of HPX into a PVA hydrogel doped with HIF-1α and PHIs encapsulated in chitosan nanoparticles and MMP inhibitors to treat early OA. Our solution aims to provide joint lubrication, promote tissue regeneration, alleviate symptoms, and prevent further degradation. The doped HPX/PVA hydrogel will be injected intra-articularly into the diseased joint.

4.1.1 HPX/PVA

PVA is chosen as the hydrogel base, while HPX, a HA/PA+HA/PM polymer matrix, is selected as the lubricant. HA/PA (hyaluronic acid/ poly-2-acrylamide-2-methylpropanesulphonic acid) and HA/PM (hyaluronic acid/ poly-2-methacryloyloxyethyl phosphorylcholine) are hyaluronan backbone complexes grafted with brush-like lubricin-like hydrophilic polymer side

chains and amphiphilic lipid-like polymer side chains, respectively [21]. HA/PA and HA/PM inherit lubricating properties from their water-binding sulphonate and phosphorylcholine groups, respectively [21]. The sulphate groups can indirectly bind with collagen via fibronectin [22, 23], stabilising the matrix. These properties allow HPX to form a stable lubrication layer to reduce the wear of the joint cartilage when injected intra-articularly [21] and prevent further formation of chondral debris due to lubrication dysfunction. While HA/PA or HA/PM alone can reduce friction, HPX can mimic the friction coefficients of human synovial fluid [21].

4.1.2 Chitosan Nanoparticle-Encapsulated HIF-1α

HIF-1α (hypoxia-inducible factor 1-α) is a transcriptional factor that regulates the growth cycle and homeostasis of chondrocytes [24], and its stabilisation suppresses chondrocyte hypertrophy [25]. The underproduction of HIF-1α can lead to apoptosis and autophagy suppression [26], making it a significant contributing factor for OA progression, given a normal hypoxic condition. If HIF-1α can be increased, hypoxia-induced apoptosis can be blocked and inhibit mitochondrial dysfunction [27]. Therefore, introducing chitosan nanoparticle-encapsulated HIF-1α via the hydrogel can supplement the supply and curb OA progression.

4.1.3 Chitosan Nanoparticle-Encapsulated Prolyl Hydroxylase Inhibitor

PHD (prolyl hydroxylase) is a proteasome responsible for the prolyl hydroxylation pathway, which is the primary degradation pathway for HIF-1α [28]. By incorporating 1,4-DPCA (1,4-dihydrophenonthrolin-4-one-3-carboxylic acid), a competitive PHI (PHD inhibitor), into the hydrogel, HIF-1α can be stabilised, and its degradation slowed [28, 29]. This allows the HIF-1α released from the nanoparticles to be more long-lasting and prolong the life of HIF-1α naturally synthesised by the body. However, 1,4-DPCA is hydrophobic [30], and must be bundled in chitosan nanoparticles to integrate the inhibitor effectively.

4.1.4 MMP Inhibitors

In OA, chondrocytes lose their phenotypic stability and express ECM-degrading MMPs (matrix metalloproteinases), with MMP13 being the significant contributing collagenase [31]. ECM remodelling causes cartilage thinning and inflammation, leading to the symptoms of OA.

Compound 24f (4-{(R)-carboxy-[5-(4'-ethoxyphenyl)-thiophene-2-sulphonylamino]-methyl}-piperidine-1-carboxylic acid isopropyl ester) is a carboxylic acid-based competitive MMP inhibitor that not only inhibits MMP13, but also other MMPs like MMP3, 9, 13, and 14, without significantly inhibiting MMP1 or TACE (TNF-α converting enzyme) [32]. Incorporating 24f can protect the joint long-term by preventing further degradation and allowing tissue healing.

4.2 Advantages

4.2.1 Superior Mechanical Strength, Lubrication, and Wear Resistance

While PVA (polyvinyl alcohol) hydrogels have good biomimetic properties, they have poor mechanical strength and friction coefficients compared to natural cartilage and low wear resistance with 36.5% weight loss after 2000 cycles [14]. However, these problems can be mitigated if lubricative polymers are added to the PVA [14, 33].

When 5% of HA/PA and 1% of HA/PM by weight are mixed into PVA, forming A5M1, the wear can be reduced three-fold, and friction by ~30%; moreover, the compressive modulus (325.3 kPa) of A5M1 is 12% higher than that of PVA, putting it within the range of natural human cartilage, while the post-load recovery is higher than natural cartilage [14].

HPX/PVA hydrogel is thus superior to PVA in terms of mechanical strength, lubrication, elasticity, and wear resistance while maintaining its biomimetic capacity, making it preferable for our application.

4.2.2 Long-Term Responsive Osteoarthritis Suppression

Despite the utility of HIF-1α as mentioned above, it only has a half-life of five to ten minutes when exposed to proteases like PHD [34], which is the case in OA, making it a poor candidate for a long-term solution conventionally.

By encapsulating the HIF-1α in nanoparticles made with chitosan, a chitin-derived polymer, its half-life can be significantly increased [35], increasing long-term effectiveness. This encapsulation allows us to sidestep the transiency of HIF-1α brought on by rapid degradation, opening the possibility of becoming a feasible candidate for a long-term treatment solution.

Chitosan is highly soluble in acidic conditions due to its protonated amino groups ($pKa < 6.05$) [36]. If OA progresses, the pH of synovial joint liquid will decrease to be as low as 6.0 [37], which can trigger the acidic hydrolysis of the chitosan nanoparticles and increase the release of HIF-1α. Hence, it can also be a responsive therapeutic agent to OA progression.

4.2.3 Chitosan Nanoparticle-Encapsulated Prolyl Hydroxylase Inhibitor

In addition to overcoming the hydrophobicity of 1,4-DPCA, as mentioned above, bundling the inhibitor in chitosan nanoparticles also allows us to control the ratio between 1,4-DPCA and HIF-1α and release them simultaneously to increase the effectiveness of both species.

4.3 Feasibility

4.3.1 Chitosan Nanoparticles

The nanoparticles can be crosslinked with sulphonate groups [38] and phosphorylcholine groups [39], which makes it very soluble in the gel and thus binds to the desired tissue in the OA joint. As mentioned above, this property of chitosan nanoparticles can also be harnessed to overcome problems HIF-1α and 1,4-DPCA suffer from and integrate them into the hydrogel.

4.3.2 Compound 24f

Since ECM remodelling by MMPs is a chronic change, they must be inhibited over a long time to allow tissue regeneration for this strategy to be effective. 24f has a very low dissociation constant (MMP13 K_i = 0.19 nM), making it a pseudo-irreversible inhibitor, allowing it to have a longer duration of action and be effective even in lower concentrations [32]. Moreover, 24f is carboxylic acid-based and hydrophilic. 24f is a feasible choice of inhibitor for our application.

4.4 Limitations

HIF-1α has pivotal importance in promoting chondrocyte phenotype, regulating ECM, and playing a cytoprotective and death-promoting role in OA [40]. Its widespread role implies that any change could cascade to affect multiple pathways, including *SOX-9*, type II collagen, and aggrecan expression [41]. The dose and concentration of HIF-1α must be delicately controlled.

Chitosan nanoparticles may induce toxicity regarding organ damage and cardiotoxicity if they migrate to unintended recipient organs [42]. Although difficult, this issue can be solved by controlling the size of nanoparticles to ensure they will not mistakenly travel to other locales.

5 Teamwork

5.1 Individual Contributions

Name	**Contributions (Report & Presentation)**
AU Wai Tak, Wales	Current Technologies
CHAN Cheuk Ka	Solution, Solution Research
HO Yu On	Current Medical Needs, Solution, Novel Ideas
WAN Chun Kit	
LAM Chi Ho	Current Technologies

5.2 Reflection

Our team has collaborated sufficiently and effectively to produce a satisfactory report.

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