1 Background

First of all, what is osteoarthritis (OA)? In this diagram, we can see a normal joint and joint suffereing from osteoarthritis. The most notable problem is the thinned cartilage and inflammation in joint, leading to bone ends rub together.

Besides, osteoarthritis like cancer that we can separate it into different stages showing its severity.

In a nutshell, osteoarthritis occurs if our cushion, cartilage, gradually deteriorates. It worsens over time, resulting in chronic pain. Like here.

And prevalence of OA increased in ageing population. Which is a 10-fold increase in 80-85, compared to 50-55 aged group. And owing to ageing population, across the globe, more and more cases will occur. According to WHO, 528 million OA cases recorded in 2019, it is an increase of 113% compared to 1990. In 2050, it is expected that OA cases may hit 1 billion across the globe.

Here is the overview for OA progression. Most of the cases are because of ageing, and then tissue damage. Phenotypic destabilisation of chondrocytes, hypertrophy, MMP produciton. And most notable in our medical imaging, cartilage thinning can be observed and eventually OA.

2 Motivation

Next, let us talk about the motivation behind our project.

The motivation of our solution comes from the 4 major problems surrounding OA that we have found.

Which is joint lubrication, ⬚ chondrocyte hypertrophy, ⬚ cartilage thinning, and ⬚ slow cartilage regeneration.

To target each of these 4 problems, we aim to investigate biomaterials that can

1. alleviate symptoms,
2. slow OA progression,
3. protect against further damage,
4. and most importantly, exert a long-term therapeutic effect so the body can heal naturally.

There are also a few requirements that the biomaterial must fulfil.

It must be mechanically strong enough and elastic enough to support the loads that joints will face. And ideally, the properties should closely mimic human cartilage.

Then, it must be able to integrate with the tissue to anchor itself in-place.

And since we aim to provide a long-term solution, the biomaterial must also be resistant to wear and tear.

However, looking at the current technology, we can see that they are each lacking in one way or another, which make them not suitable for long term use.

We aim to provide a solution that can improve upon the current technologies and encompass all the necessary criteria.

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3 Solution

Next, let us talk about our proposed solution.

Recall that we have raised 4 problem focus that we want to tackle.

Our solution roughly consists of 4 aspects that can tackle these problems individually.

For the first focus of joint lubrication, we used HPX polymers in our solution.

HPX is a mixture of two hyaluronan backbone polymers, HA/PA and HA/PM. HA/PA has brush-like hydrophilic polymer side chains that act like lubricin grafted onto the HA backbone, while HA/PM has amphiphilic lipid-like polymer side chains grafted onto the backbone.

Both of them have lubricating properties. HA/PA gets its lubricating properties from its sulphonate groups, and HA/PM gets them from its phosphoryl-choline groups. So we will use them for our joint lubrication.

The reason for using two polymer species for lubrication is that they have better lubricating properties when mixed together compared to using either one of them alone.

For our application, we will use PVA as a hydrogel base and add HPX to make A5M1.

A5M1 stands for 5% HA/PA and 1% HA/PM by weight. And the rest is just PVA.

Pure PVA has been investigated for cartilage repair, but it is not strong enough and not elastic enough, it has too much friction, and it suffered a 37% weight loss after 2000 cycles.

But if we incorporate HPX into PVA, the friction and compressive modulus can mimic natural synovial fluid and cartilage. The material can recovery better after being subjected to loads. Notably, the wear resistance also increases by 70%.

Additionally, the sulphate groups found in HA/PA can indirectly bind with collagen via fibronectin, which helps anchor and stabilise the matrix to facilitate joint articulation.

By lubricating the joint, we can decrease friction to minimise tissue damage caused by rubbing. And in turn prevent the formation of chondral debris. Which, consequently, reduce pain and inflammation.

Next, to stabilise the chondrocyte phenotype, we used HIF-1α and PHD inhibitor.

HIF-1α is a transcriptional factor that regulates the growth cycle and homeostasis in chondrocytes. Importantly, it also suppresses chondrocyte hypertrophy, which is a common cause of OA.

In OA cases, we can observe an underproduction of HIF-1α. And that causes chondrocyte apoptosis, mitochondrial dysfunction, hypertrophy, which cascades into OA symptoms and OA progression.

So by introducing HIF-1α, we can stabilise the chondrocytes, return them to homeostasis, and therefore prevent the increase in OA severity.

However, there is one problem.

HIF-1α is rapidly degraded via prolyl hydroxylation, which makes it only have a half-life of 5-10 mins, which is not good in terms of long-term efficacy.

So we thought to also include a PHD inhibitor to stabilise the HIF-1α we added, in addition to prolonging the natural HIF-1α produced by the body.

Both of these will be encapsulated in chitosan nanoparticles.

Chitosan nanoparticles serve multiple functions in our application.

It can shield the HIF-1α from enzymes prior to release. It helps 1,4-DPCA to overcome hydrophobicity so that it can be integrated into the hydrogel.

Furthermore, since they are bundled together, we can control the ratio between them to ensure optimal effectiveness.

Another important utility of chitosan nanoparticles is that they hydrolyse more readily under acidic conditions, which is great for our application since the ECM becomes more acidic when OA progresses. This means that the nanoparticles can help release the drugs responsively based on the OA severity.

For the integration, chitosan nanoparticles can crosslink with HA/PA. This allows us to inject the nanoparticles intra-articularly together with the HPX/PVA.

For ECM protection, we decided to add an MMP inhibitor.

In OA, hypertrophic chondrocytes release a family of enzymes called MMPs. They are responsible for ECM degradation and cartilage thinning, which causes the symptoms. Out of the MMPs, 13 is the major player in OA.

So we also add an MMP inhibitor called 24f to protect the cartilage from further degradation and thinning.

24f is a competitive inhibitor of MMP-13. Although 13 is the major target, it can also inhibit MMP-3, -9, and -14, which are also related to OA. And it can do these without affecting MMP-1 and TACE, which are important for other functions.

Recall that we want a long-term solution, which is partly why we chose 24f. It has a very low dissociation constant, which makes the inhibition pseudo-irreversible. This means it can be effective at lower concentrations and the effects can be longer-lasting.

By protecting the cartilage from further damage, we can allow the slow natural regeneration process to take place.

Here is a brief recap of our solution.

First, we used PVA as a hydrogel base since it is biocompatible. Then we incorporate HPX polymers into the gel to lubricate the joint.

Then, we have HIF-1α and PHD inhibitor to stabilise the chondrocyte phenotype to prevent OA progression and reduce the severity.

Then, we have 24f to protect the cartilage by inhibiting matrix-degrading enzymes to allow natural healing.

Finally, we encapsulate the drugs in chitosan nanoparticles to integrate them into the hydrogel. And since the drugs are bundled, we can also control the ratios better. The acidic hydrolysis of the nanoparticles also allows the drug release to be responsive to OA progression.

All of our choices revolve around make a long-term solution for OA.

Since OA is chronic, and cartilage healing is slow due to its avascular nature, only long-term solutions can improve the patient’s quality of life.

That is why we chose a durable and strong hydrogel base. And we chose drugs that stop further damage and encourage natural regeneration.

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4 Discussion

Our incorparation of chitosan NPs into HPX/PVA. There are several proof for the effectiveness of NPs and HPX/PVA

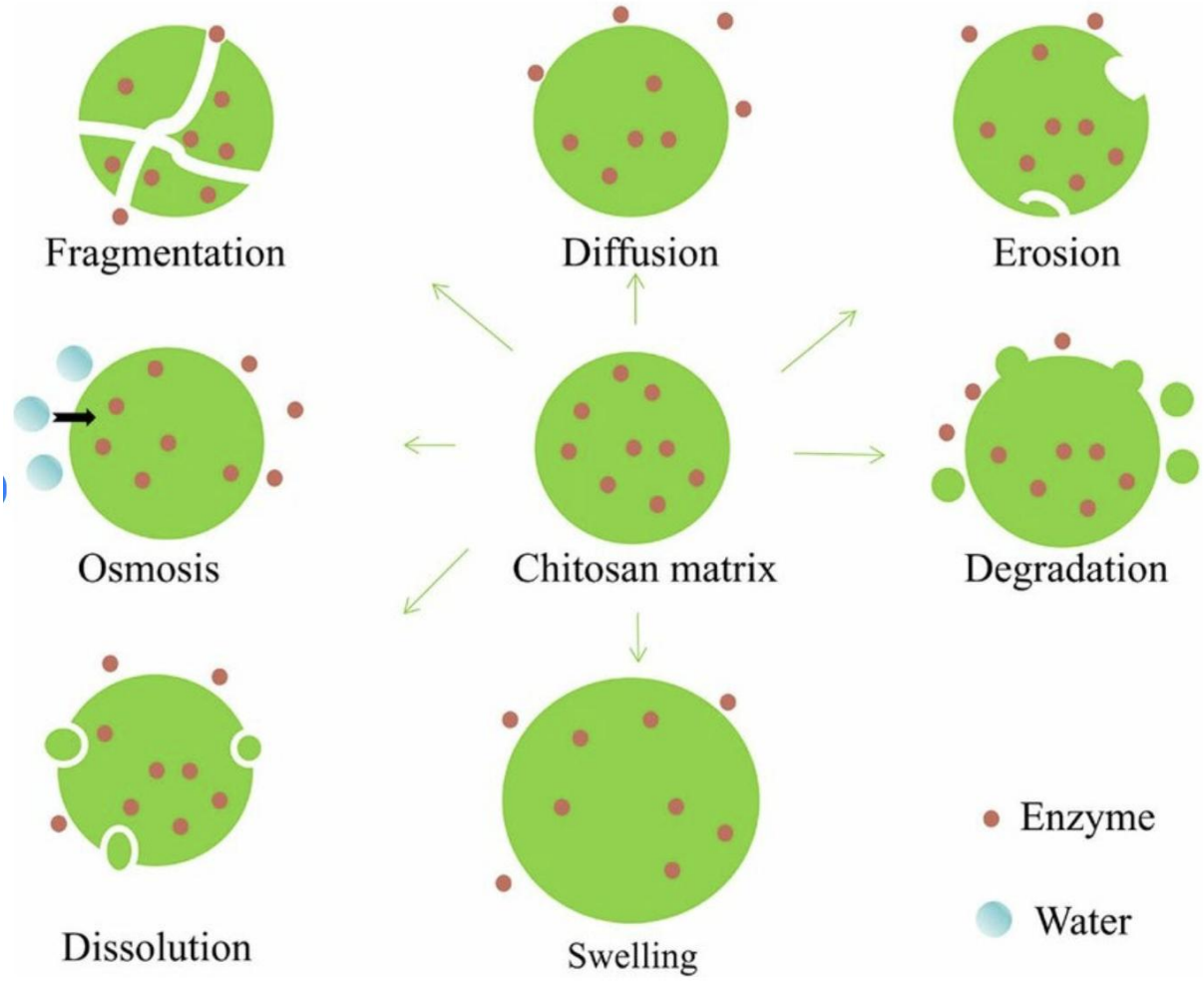
And the NP can be gradual hydrolysis to release the drugs, providing a long-term and up-to-dated effect to the situation

There are some drawbacks, like HIF-1a is not only an inhibitor of OA. It is found that high concentration of HIF-1a may lead to adverse effects for OA. When it comes to the use of NPs, it may cause cytotoxicity and unintended effects, size of it has to be controlled

Fact Sheet for NPs synthesis

NPs: Reversed micelles method, used precipitation methods. Using a solution with chitosan nanoparticle and oven-drying with TIP → wet synthesis

And we can have a dry synthesis, using spray drying, it is acid-free method and solvent-free method despite it is time-consuming



How to stain cytokine (ELISA, HPLC, and bioassay)

Harvest the cells and stain cells with a corresponding cocktail of fluorescently labelled antibodies for 30 min at 4°C. Wash the cells twice with PBS plus 2% FCS (PBS-F). Fix the stained cells with 250 µl of Cytofix/Cytoperm solution (BD Biosciences) or 2% paraformaldehyde for 20 min at 4°C.