Preparation of Hydrogel Scaffold for Oesteoarthritis

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ABSTRACT
The main objective of this study is developing a hydrogel scaffold using Poly Vinyl Alcohol(PVA) and Gelatin for Osteoarthritis by repeated freeze-thawing method and to examine the characterestics of hydrogel such as swelling ability. This smooth and porous hydrogel scaffold were successfully prepared by three cycles of freeze-thawing method and lyophilization.The morphology of the hydrogel scaffold were characterised by Scanning Electron Microscope(SEM) and the crossover of the gel was understood by using rheological study.

INTRODUCTION
Osteoarthritis (OA) is most common type of arthritis or degenerative joint disease
and it is the highest-ranking disease among the musculoskeletal diseases.Worldwide statistics indicates that 9.6% of men and 18% of women ≥ 60 years have symptoms of OA. It is a progressive musculoskeletal
disorder caused by gradual loss of articular cartilage [1,3]. The osteochondral complex constituted majorly by water, collagen type I, and hydroxyapatite (HA) which provides
the tissue's stiffness and compressive strength [1]. The differences in morphology, composition and mechanicalproperties of bone and cartilage indicate the complexity of bone-cartilage tissue interface. Traumatic or degenerative causes lead to osteoarthritis of the articular cartilage characterized by cartilage loss, subchondral bone thickening, and osteophyte formation. The initial morphologic changes include thinning and fragmentation of cartilage followed by continuous loss and fibrosis of articular cartilage that fails to protect the underlying subchondral bone. The cartilage once damaged fails to heal spontaneously. In the early stage of OA, medication and physical therapies are used as conservative treatments for the purpose of reducing pain and to delay the progressive structural deterioration in affected joints. However, severe degenerative conditions require surgical interventions such as microfracture, autologous chondrocyte implantation (ACI), osteochondral autograft transfer, joint replacement and osteotomy (OAT) in order to restore tissue function[5]. These treatment modalities do not completely restore the native function of articulating cartilage as the tissue formed is composed of collagen I which is inferior to collagen II. Therefore, tissue-engineering strategies have been developed to cartilag Among several biomaterials, hydrogels are widely used as a potential artificial articular cartilage substitutes due to their structural similarity to cartilage [10]. Hydrogels are biocompatible, three-dimensional porousstructures that permit tailorability of physicochemical and biological characteristics. Hydrogel prepared by freezing and thawing techniques shows an increased mechanical strength due to the existence of crystalline regions that serve as physical crosslinks[10]. An ideal scaffold for repair of osteochondral injury necessitates to regenerate and facilitate the restoration of both cartilage and subchondral bone simultaneously. Hence, in this study, biphasic scaffold by freeze-thaw technique has been designed by constituting glycosaminoglycan in the chondral layer (articular cartilage) and nanohydroxyapatite in the underlying calcified tissue (subchondral bone).

CHOICE OF MATERIAL
Polyvinylalcohol(PVA)
Polyvinyl alcohol(PVA) is a water-soluble synthetic polymer. It is used in papermaking, textiles, and a variety of coating. It is white colourless and odourless. It is sometimes supplied as beads or as solution in water. PVA is used as an emulsion polymerization aid, as protective colloid, to make polyvinyl alcohol dispersions. This is largest market application in china. The other uses of PVA are

injection moulding of soluble containers for active release of detergents and agrichemicals. Paper adhesive with boric acid in spiral tube winding and solid board production, thicker, modifier, in polyvinyl alcohols glues. Textile sizing agents, paper coating, release liner. As a water-soluble film useful for packaging. An example is the envelope containing laundry adult incontienence products as a biodegradable plastic backing sheet. As a film used in the water transfer printing process. Used in eye drops (such as artificial tears to treat dry eyes) and hard contact lens solution as a lubricant. PVA fibre, as reinforcement in concrete. Raw material to polyvi in Doppler flow testing. nyl alcohol and ester of nitric acid and polyvinyl alcohol. As a surfactant for the formation of polymer encapsulated nanobeads. Used in protective chemical-resistant gloves. Used as a fixative for specimen collection, especially stool samples. When doped with iodine, PVA can be used to polarized light. As an embolization agent in a medical procedures. Carotid phantoms for use as synthetic vessels

GELATIN

Gelatin is a translucent, colourless, brittle, flavourless food derived from collagen obtained from various animals body parts. It is commonly used as a gelling agent in food, pharmaceutical drugs, vitamin capsules, photography, and cosmetic manufracturing.

Substances containing gelatin or functioning in a similar are called "gelatinous''. Gelatin is a irreversibly hydrolysed form of collagen, were in the hydrolysis results in the reduction of protein fibrils into smaller peptides which will have, broad molecular weight animals such as domesticated cattle, chicken, pigs, and fish. During hydrolysis, the natural molecular bones between individual collagen strands are broken down into a form that rearranges more ranges associated with physical and chemical methods of denaturation, based on the process of hydrolysis. It is found in most gummy candy, as well as other products such as marshmallows, gelatin desserts, and some ice creams, dips, and yogurts. Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissue of easily. Its chemical composition many aspects, closely similar to that of its parent collagen.

METHODS

The polymer is dissolved in the distilled water. It is made into a solution of 8:2 ratio of PVA and gelatin in 20ml of distilled water by using Magnetic Stirrer . Magnetic Stirrer or magnetic mixer is a laboratoric device that employs a rotating magnetic field to cause a stir bar immersed in a liquid to spin very quickly ,thus stirring it. The left knob of the magnetic stirrer controls the heat and the right knob controls the stirring rate. Pour the solution in a petridish and applying the Freeze-Thaw method.

FREEZE-THAWING

The hydrogel was prepared by three repeated cycles of freeze thawing method.Freeze thaw study was carried out by subjecting the hydrogel scaffold to freezing for 24hrs in a deep freezer followed by thawing for 3hrs at room temperature.Freeze thaw study is based on the principle that excipient cannot protect the nanoparticle during the first step of freezing durng lyophilization.It is not likely to be an effective cryoprotectant.The aim of the present study is preparation of polymeric nanoparticle of PVA.When the hydrogel freezes it expands and produce pressure in the pores of the hydrogel.The major purpose of Freeze Thaw method is to enhance the stability of hydrogel.It increases porosity of hydrogel that regenerates the chondrocytes in the region of cartilage.This method also produces high durability to the gel.

(a) Poly vinyl Alcohol (b) Gelatin (c) Magnetic stirrer (d) ,(e) A solution 8:2 ratio of PVA

and gelatin in 20ml of distilled water is placed on the magnetic stirrer. (f) Formation of hydrogel after the second cycle of freeze thaw method

RESULTS AND DISCUSSIONS

SWELLING STUDY

A cross linked polymer hydrogel swell but not dissolved in water or a solvent enters it.The swelling properties, which usually use degree of swelling to define hydrogels,depend on many factors such as network density, solvent nature , polymer solvent, interaction parameter.The properties of water swelling of hydrogel were studied in this work.

The water uptake profile of the hydrogel was assessed by immersing the hydrogel scaffold in Simulated Body Fluid (SBF) at ambient temperature(37ºC).At the different time interval of 15,30,45,60 and 120 mins, the scaffold were removed from the buffer and weighed.The swelling ratio was calculated using the following equation:

Swelling ratio(%) = (Ws-Wd)/Wd * 100

Ws : Weight of the swollen hydrogel

Wd : Weight of the dried polymer

RESULTS:

The swelling percentage of hydrogel scaffold in various time duration has been shown in a table given below. As per the equation ,

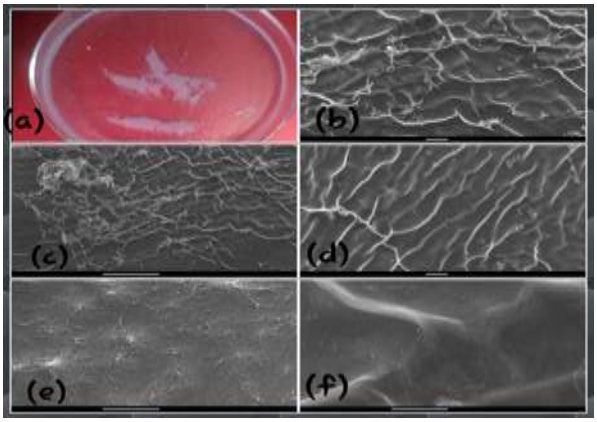
Here, Wd = 0.4g

By taking Wd as a constant upto 120 mins,the swelling percentage is calculated with different values of swollen hydrogel. At the time duration 15 mins , Ws=0.46g and thus the swelling percentage is obtained as 14.83%. Similarly, different swelling percentage in various time duration are calculated and shown in the table below.

Time in Mins	Ws	Swelling ratio
15	0.46	14.83
30	0.5	23.57
45	0.52	30.20
60	0.54	34.95
120	0.56	37.96

SCANNING ELECTRON MICROSCOPE

The surface morphology for porosity of PVA/Gelatin were analaysed by SEM.The microscopic images of hydrogel is given below:

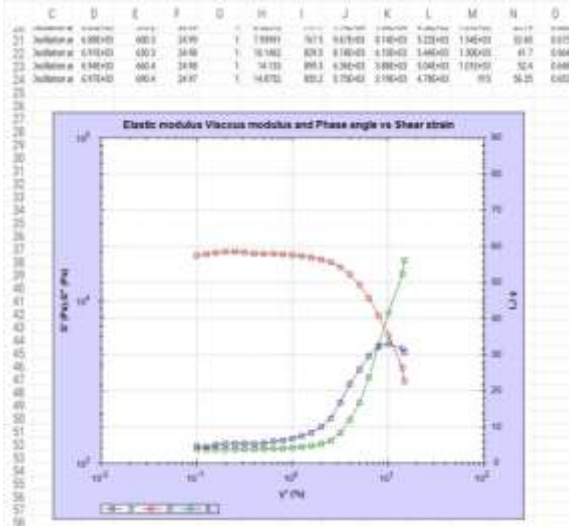


(a) image of hydrogel in a petridish

(b),(c),(d),(e),(f) microscopic images of hydrogel

RHEOLOGICAL STUDY :

Along with swelling study and blood compatibility mechanical properties are also crucial importance of hydrogel scaffolds. Hydrogels are usually soft materials, which may benefits from the incorporition or inorganic particles,not only due to the acquired bioactivity, but also due to improve mechanical property. They exhibits complex visco elastic property which can be evaluated in various ways. In this study , the elastic modules, viscous modules, phase modules of hydrogel against shear strain is considerd which is denoted as G", G' and δ respectively. Phase angle represent the visco elastic property of gel. Increasing strain percentage was applied over the gel around the 0.1-100%. The graphical representation of this study is given below.



ELASTIC MODULUS

Elastic modulus of gel slowly increases and then decreases linearly with the applied strain upto 20%.

VISCOUS MODULUS

The viscous modulous of hydrogel is constant upto 5% of applied strain and gradually decreases.

PHASE ANGLE

Phase angle of the gel is seen to be constant upto 2% of the applied strain and exponentially increases with increasing applied shear strain.

To understand the gel cross over, increasing strain percentage was shear applied over the gel(0.1% - 100%).

Approx.at 12%, gel crossover happened.

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