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Physical Characterisation and In-Vitro Kinetic Modelling on Drug Release Study of Polyvinyl Alcohol / Polyethylene Glycol / Pectin Hydrogel for Cartilage Tissue Application

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The treatment of articular cartilage tissue injuries due to trauma or overuse is a challenging task in the medical science of orthopedics. The hydrogel's potential as multifunctional cartilage tissue grafts has often been an interesting topic to study because of the capability of hydrogel to swell and possess the mechanical behavior required as a cartilage substitute. This work reports the development of polyvinyl alcohol (PVA) / polyethylene glycol (PEG) / pectin hydrogel using the freeze-thawing technique. Mechanical and hydrophilic properties of the hydrogels were optimum at 5 wt% of pectin loading with 0.109±0.023MPa of young modulus, 0.032±0.011MPa compressive strength, and 96.80±1.5% swelling percentage. The controllable drug release studies used methylene blue (MB) as a drug model to demonstrate kinetic release mechanisms. Studies from Peppas-Korsmeyer Kinetics Model, Higuchi Kinetics Model, and Zero-Order Kinetics Model indicate that drug release kinetics follows Fickian's Diffusion theory. The incorporation of pectin provides a wide area for drug entrapment and changes the swelling kinetic properties that affect the release mechanism of the hydrogel. These are a good indication for PVA/PEG/Pectin hydrogel as a new potential drug delivery carrier in the future of articular cartilage tissue repair.

Keywords: polyvinyl alcohol; polyethylene glycol; pectin; tissue engineering; cartilage

I. INTRODUCTION

The regeneration of articular cartilage tissue is a challenging duty in the orthopaedic fields. Articular cartilage is the smooth, white and thin layer of tissue covering joint surfaces (Chuang *et al.,* 2018). This special tissue can absorb high impact and shock to protect bone tissue (Censi *et. al.,* 2015; Eslahi *et al.,* 2016). Trauma or misuse of the tissue, on the other hand, can result in cartilage damage or breakdown (Walker & Maihally, 2015). Unfortunately, injured cartilage's ability to self-heal is restricted (Vilela *et al.,* 2015). Thenceforward, the treatment for cartilage tissue damage growing up which are micro-fracture (Mithoefer *et al.,* 2005), osteochondral (Bently *et al.,* 2012), autologous (Brittberg *et al.,* 1994) and total joint replacement (Foran *et al.,* 2013). However, each of these treatments has its restraint (Kreua *et al.,* 2006). Osteochondral and autologous have a high risk of differences in cartilage structure, slow recovery and failure to generate tissue that adequately restores damaged cartilage (Vega *et al.,* 2017). For the sake of growing demand in the cartilage regeneration treatment, these drawbacks announce an

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increasing prerequisite for alternative techniques (Moriarty *et al.,* 2016). A biomaterial-based tissue engineering strategy is a high potential to regenerate articular cartilage, including the use of hydrogels (Broom & Oloyede, 1998). The cartilage tissues are made up of a long-chain polymeric material that swells more than the aqueous phase, resulting in hydrogel-like behaviour (Kuo & Ma, 2001).

Hydrogels are polymeric materials that contain pores that are large enough to accommodate living cells, have the ability to form multifunctional cartilage grafts since they are capable of physiological swelling (Chung *et al.,* 2013), and have enough mechanical behaviour required for this use as articular cartilage substitutes (Chuang *et al.,* 2018). The drawback of the osteochondral treatment can be solved by hydrogel because they can produce structural similarity to the extracellular molecule (ECM) of cartilage tissue (Frenkel & Di Cesare, 2004). Therefore, biomaterials selection in hydrogel plays an important role that acts as artificial cartilage substitutes and encourages the renewal of newborn tissue (DeMerlis & Schoneker, 2003).

In this study, the material selection for hydrogel is Poly(vinyl) alcohol (PVA) polymer because it is a hydrophilic polymer that contains many hydroxyl groups and can form hydrogen bonds with free water molecules (Sriamomsak, 2003). However, PVA has limitations in cell attachment of the site and the mechanical strength. Because of these drawbacks, the bioactive polymer was selected to help PVA in cell attachment is pectin. Pectin is a complex mixture of polysaccharides located in the middle of the lamella of the cell wall in all plants (Harris, 1992). The mechanical strength of PVA and pectin polymer can be increased by undergoing gelation process with help from poly(ethylene) glycol (PEG) to strengthen hydrogel structure. PEG is a hydrophilic oligomer or polymer synthesised from ethylene oxide (Guan *et al.,* 2015). PEG will undergo the PEGylation process to combine with pectin and PVA polymer to strengthen the structure of hydrogel (Derakhshanfar *et al.,* 2018). PEG's properties make it a promising biomaterial for implanting into injured tissue or bone to facilitate cell-based regeneration and repair under strict supervision (Kobayashi & Oka, 2004). Though few reports on the importance of hydrogels in tissue engineering, this is the first study that used PVA, PEG, and Pectin as a biomaterial for hydrogel specifically for cartilage tissue repair. The properties of the hydrogel were evaluated by compression test and hydrophilicity (degree of swelling). The purpose of this research was to produce a PVA/PEG/Pectin hydrogel with mechanical and hydrophilic qualities similar to natural cartilage while also allowing for effective drug load transfer and release to aid in cell recruitment for cartilage regeneration.

II. MATERIALS AND METHOD

A. PVA/PEG/Pectin Hydrogel Preparation

The PVA/PEG/Pectin hydrogel was prepared by dissolving 7.48g PVA powder (BIS chemical) into 50mL distilled water at 100^{o}C using magnetic stirred at 300rpm until gelatinization. Then, 0.013g PEG pallet (BIS chemical) was added into PVA solution and continued stirred until homogenize. After that, pectin powder (BIS chemical) (1%, 3%, 5%, 7%, 9%, and 11%) was added into PVA/PEG solution until homogenize solution formed and the solution was put into the silicon mould. The polymer solution underwent a freeze-thaw process at -20^{o}C and room temperature for 24 hours for 5 times (Kobayashi & Oka, 2004; Derakhshanfar *et al.,* 2018).

B. Mechanical Testing: Compression

Compression test analysis (TecturePro CT V1.8 Build 31) was conducted to investigate the mechanical integrity of the hydrogel by applying the 10kg load cell. The young modulus and the stress limit of the hydrogel were also obtained from this testing. Before testing, the temperature was set during at` 575^{o}C with a speed of 0.5 mm/s. The young modulus was calculated using Equation (1):

$$\text{Young modulus (MPa)} = \text{stress (MPa)} / \text{strain} \quad (1)$$

The value for stress and strain was obtained from the compression graph from the test (Gaharwar *et al.,* 2011).

C. Degree of Swelling

Swelling testing analysis was conducted by soaking the hydrogel (1cm x 1cm) in distilled water for 24 hours to study the ability of the hydrogel to absorb water and swell. The

weight before and after the testing was measured by using an analytical weight balance. The degree of swelling was calculated by using Equation (2):

$$DS\ (\%) = (Ws - Wd) / Wd \times 100 \quad (2)$$

Where Wd is the weight before, and Ws is the weight after soaked into distilled water. This testing was run 3 times to get the result (Chang *et al.,* 2010).

D. Drug Loading and Kinetic Model Drug Release

The test was carried out on specimens with a surface area of 0.25cm^2 before soaked into 25ml of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% methylene blue solution for 24 hours. After 24 hours, the weight and the size of the samples were measured. After that, these samples were soaked into potassium buffer saline to study the drug release of the hydrogel. To investigate the drug release of the hydrogel, researchers used buffered saline. UV spectroscopy (Thermo Scientific, Genesys 10S UV-VIS) on the release media at 260nm at time intervals of 2, 4, 6, 12, and 24 hours was used to determine drug release. The mechanism is also being studied with several kinetic models (Bently *et al.,* 2012):

Zero-Order Kinetics Model

$$Q_t = Q_0 + K_0 t \quad (3)$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of medicine in the solution, and K_0 is the zero-order release constant expressed in the units of concentration/time.

Higuchi Model

$$Q = K_H + t^{1/2} \quad (4)$$

Where Q is the amount of drug released in time, t and K_H is the Higuchi dissolution constant. Therefore, the data obtained were plotted as cumulative percentage drug release versus square root of time.

Korsmeyer-Peppas Model

$$M_t/M_0 = Kt^n \quad (5)$$

Where M_t/M_0 is a fraction of drug released at time t, k is the release rate constant, and n is the release exponent. The n value is used to differentiate the release mechanism for cylindrical shaped matrices.

III. RESULT AND DISCUSSION

A. Compression Test

The mechanical characteristics of the implant for cartilage tissue engineering are critical factors owing to the effect of the force that can be felt from neighbouring tissues in vivo. When a pressure differential is provided, the mechanical reaction of cartilage is tightly linked to the movement of fluid through tissue. The hydrogel's ultimate compressive strength and young modulus are shown in Table 1.

Table 1. Young Modulus and ultimate compressive strength

Sample	Ultimate compressive strength (MPa)	Young modulus (MPa)
PVA	0.014588 ± 0.01	0.050303 ± 0.01
PVA/PEG	0.015225 ± 0.01	0.052500 ± 0.01
PVA/PEG/Pectin 1%	0.021341 ± 0.01	0.076217 ± 0.01
PVA/PEG/Pectin 3%	0.028540 ± 0.01	0.098413 ± 0.01
PVA/PEG/Pectin 5%	0.031597 ± 0.01	0.108955 ± 0.01
PVA/PEG/Pectin 7%	0.044912 ± 0.01	0.154868 ± 0.01
PVA/PEG/Pectin 9%	0.048606 ± 0.01	0.167606 ± 0.01

The addition of pectin to the polymeric system increased the values of Young's modulus and stress at the limit. The higher the proportion of pectin in the hydrogel, the greater the compressive strength since the pectin filled the hydrogel's empty structure. Moreover, the samples with a low percentage of pectin concentration had lowwith a low percentage of pectin concentration had low compressive strength, resulting in poor crosslinking condition.

As a result, including pectin into the hydrogel can increase mechanical qualities. In comparison to previous hydrogels

proposed for cartilage regeneration, this hydrogel possesses exceptional compressive characteristics. Studies reported on PEG/nano-silica combination ed compressive stress of 0.05–0.066 MPa, which is 2-fold lower than hydrogel in this study (Gaharwar *et al.,* 2011). The other research developed hyaluronic acid/PEG hydrogel for cartilage tissue with 0.027 MPa and 0.1094 MPa for compressive modulus and stress at limit, respectively, which is a 6-fold decrease compressive properties compared to PVA/PEG/Pectin hydrogel (Chang *et al.,* 2010). As shown in Table 2, the compressive young's modulus of PVA/PEG/pectin was closed to the healthy human articular cartilage.

Table 2. Natural human cartilage biomechanical properties and cartilage tissue engineering constructs

Mechanical properties	Human cartilage	Engineered Cartilage	PVA/PEG/pectin hydrogel
Compression young's modulus (MPa)	0.2-0.85 (Mow & Guo, 2002)	0.005-5.9 (Bryant *et al.,* 2004)	0.167 ± 0.01
Complex shear modulus (MPa)	0.2-2.0 (Zhu *et al.,* 1993)	0.023-0.11 (LeRoux *et al*, 1999)	0.048 ± 0.01

B. Degree of Swelling

One of the most important aspects of synthetic hydrogel biocompatibility is their capacity to absorb aqueous solutions, which confers unique physiochemical characteristics on the scaffolds (Killion *et al.,* 2014). This gives the scaffold physiological stability, biomolecule permeability, and low interfacial tension. The degree of swelling was evaluated for these purposes, as explained later—Figure 1 depicted graphs of the percentage of swelling of a hydrogel in an aqueous solution. The studies showed that PVA/PEG with 5% of pectin has the highest rate of swelling than other samples, with the percentage of swelling up to 96.8%. This phenomenon was associated with the pectin occupying the empty space within the hydrogel's pore, which would else be taken up by water (Neffe *et al.,* 2011). Hydrogels with high water content, up to 90% are viscoelastic and resemble the network structure of the natural articular cartilage (Wang *et al.,* 2012). Swelling properties is the main factors that need to be considered in order to load and release the drug effectively. If the swelling percentage is lower, the hydrogel cannot load a high amount of drug, so the drug delivery system is ineffective, and the healing rate of the injured tissue is slow.

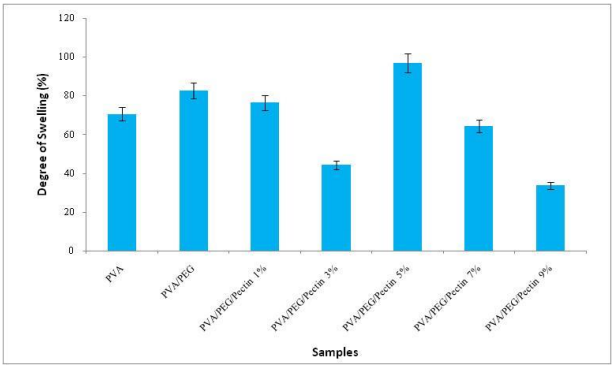

Figure 1. Swelling percentage of different hydrogel composition

From the compression test and degree of swelling, it can be suggested that the best formulation is PVA/PEG with 5% pectin due to optimal mechanical and hydrophilicity properties suitable for cartilage tissue replacement. Three candidates have a potential modulus: PVA/PEG/Pectin 5%, PVA/PEG/Pectin 7% and PVA/PEG/Pectin 9% for cartilage application. The highest young modulus for this study is 0.167 MPa but the swelling percentage is the lowest one. The highest percentage of swelling is shown by PVA/PEG/Pectin 5%. This hydrogel can swell up to 96.80% with compression young modulus 0.109 MPa that fulfils cartilage tissue's mechanical strength. The optimum formulation, PVA/PEG/Pectin 5%, undergoes drug loading and kinetic release study for future applications.

C. Drug Loading and Release

Cartilage tissue serves as a repository for lipids, collagen, and cells. However, due to its nature, it has relatively poor healing qualities. As a result, when a patient experiences joint pain, medications are typically provided intravenously in high quantities for extended periods. This can result in adverse effects such as antibiotic resistance, lengthy hospitalisation, and excessive medical costs (Soundrapandian *et al.,* 2009). To circumvent these disadvantages, localised medicine delivery is becoming

increasingly appealing. Figure 2 depicts the findings from a study of the hydrogel's capacity to load the medication.

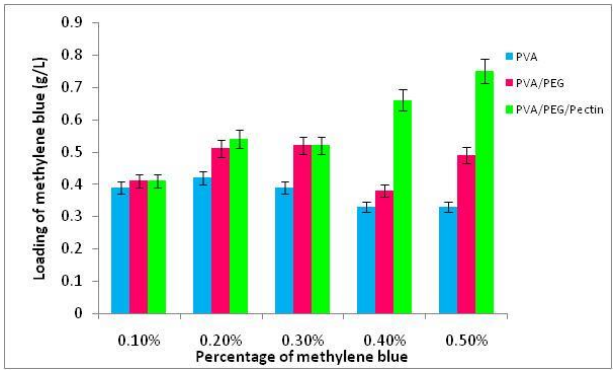

Figure 2. Loading capacity of different methylene blue concentration at PVA, PVA/PEG, and PVA/PEG/Pectin hydrogel

Figure 2 shows that the loading of methylene blue into the PVA/PEG/Pectin hydrogel is better than PVA and PVA/PEG hydrogel. This activity is because of the bioactive polysaccharide pectin that can easily attach to the methylene blue. The concentration of methylene blue also plays a big role in the drug loading capacity of the hydrogel. The graph shows the addition of pectin in hydrogel formulation has increased the uptake of methylene blue by the hydrogel, although at high concentrations. From the graph, the best percentage of methylene blue is 0.5%, with the highest drug loading up to 0.75 g/cm^2 for PVA/PEG/Pectin hydrogel when undergone through drug release test.

To assess the drug release profile using methylene blue, 0.5 % of the methylene blue in the hydrogel was selected in this investigation. Methylene blue was chosen for its antimicrobial resistance capabilities, ability to reduce the risk of post-surgery infection, and therapeutic importance in treating osteomyelitis. (Xie *et al.,* 2009).

D. Drug Release based on Kinetic Model Release

As shown in Figure 3, both PVA and PVA/PEG/Pectin hydrogel have a similar release pattern. The burst release pattern at early release for neat PVA hydrogel makes it unsuitable for drug delivery carriers. While PVA/PEG/Pectin hydrogel shows slow rate drug released compared to neat PVA hydrogel, which contributes to controllable and effective long-term drug effect. Unlike the neat PVA hydrogel, PVA/PEG/Pectin hydrogel has stronger intermolecular strength and lead to a delay in swelling. The slow swelling rate makes the drug release slower. The egg-box structure of the pectin area causes the drug to immobile and thus prevents the drug release. Besides, pectin at pH 7 is negatively charged. This will provide an intermolecular attraction between pectin and methylene blue molecules as they are cation. Therefore, it one of the reasons for the controllable drug release rate in a hydrogel containing pectin.

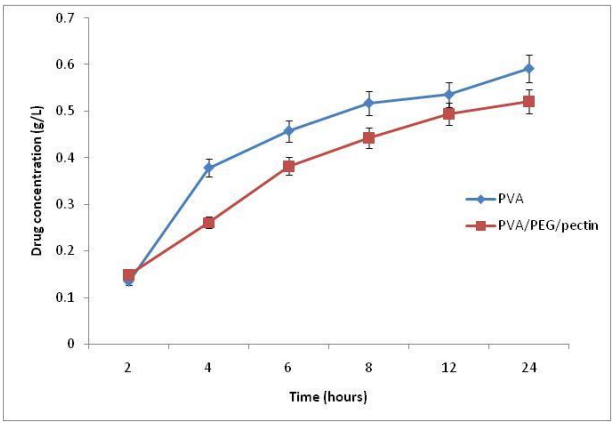

Figure 3. Drug release profile of PVA and PVA/PEG/Pectin hydrogel

Kinetic modelling of drug release was calculated using the concentration release of the hydrogel and tabulated in Table 3. PVA/PEG/Pectin hydrogel is fitted most on the Higuchi Model compared to Zero-Order. This shows that the release pattern is not constant release as stated by the perfect theory of Zero-Order. However, according to the Higuchi Model, the mechanism of drug release from the matrix follows the diffusion-controlled system. Based on Korsmeyer -Peppas theory, n=0.248 shows that the release pattern follows Fickian's diffusion theory. These theories explain that the drug release from the hydrogel matrix is influenced by the diffusion factor only.

Table 3. Zero-Order equation, Higuchi equation and Korsmeyer-Peppas equation release profile

Hydrogel		PVA	PVA/PEG/Pectin
Zero-order	R^2	0.8279	0.9409
	K_0	0.0537	0.0749
Higuchi	r^2	0.8275	0.9413
	K_H	9.4729	9.9857
Koshmeyer Peppas	**N**	0.349	0.248

IV. CONCLUSION

Incorporation of pectin in PVA/PEG hydrogel has successfully improved the mechanical and hydrophilic properties, demonstrating that this hydrogel has high potential to be used as an articular cartilage tissue graft. The drug release mechanism of the PVA/PEG/Pectin hydrogel is controlled by the swelling capabilities that affect the releasing area. The presence of pectin changes the swelling kinetics by providing strong interaction between polymer and drug that can prolong release capabilities. The Higuchi Model and Korsmeyer-Peppas result proves that the factor for the drug release is only influenced by the diffusion factor, which will help in tissue repair. Although works have been done on physical and drug release mechanism properties, further investigation is needed to study the drug release system during in-vitro and in-vivo cartilage growth for a more feasible understanding before undergoing a clinical trial.

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