Poly(vinyl alcohol)-based hydrogels for joint prosthesis

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Abstract. Cartilage is a specialized tissue responsible for mediating contact between bones on surfaces with relative movement. Its dense extracellular matrix, rich in chondrocytes and various molecules, allows a slight yield to external loads and provides a lubricated surface that lowers friction. Joint diseases such as osteoarthritis or rheumatoid arthritis affect several million people around the world, and their impact is expected to continue to increase with aging populations and rising obesity. The best treatment currently available for severe joint disease is total replacement. This involves the use of biocompatible metal alloys that articulate against other metal, ceramic or polyethylene coatings. These materials present some limitations, namely the shielding of the bone with regard to the application of forces, which can result in osteoporosis, wear leading to immunogenic debris and eventual dislocation and fracture. The biomechanical design of these implants relies on "hard-on-hard" and "hard-on-soft" interactions. This type of design does not mimic the soft-on-soft interactions that occur in natural cartilage. Hydrogels, namely polyvinyl alcohol-based hydrogels (PVA), have been studied and mentioned as a possible alternative for materials used in hip and knee prostheses because of their biocompatibility, swelling ability and tribological behavior. In this work, different formulations of PVA hydrogels were investigated in terms of their physical properties (swelling, wettability, thermotropic behavior) and mechanical properties (Young's modulus, toughness, ultimate tensile strength and maximum strain). Total replacement of the hip or knee and the results were compared with the natural cartilage and other PVA hydrogels in the literature. It was observed that the swelling and thermotropic behavior of the materials was very similar to those found in the literature. It has also been observed that, mechanically, higher molecular weight hydrogels have characteristics comparable to those of natural cartilages. However, all the gels studied have a higher compressive strength than traction, as opposed to the typical cartilage behavior, which is more resistant to compression.

Keywords: PVA hydrogels, PVP, glyoxal, articular cartilage replacement, mechanical properties, thermotropic properties, swelling, wettability

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1. Introduction

Cartilage is an avascular, specialized connective tissue composed of a dense extracellular matrix which houses chondrocytes and a variety of molecules, with a very low capacity for intrinsic healing and repair. Joint pathologies such as osteoarthritis (OA) and rheumatoid arthritis (RA) are leading causes of disability worldwide.[1,2] Obesity and old age are increasing trends in the world's demographics that are expected to intensity in their impact.[3]

In the natural joint, all articulating bones are lined with cartilage, creating a soft-on-soft interaction interface. When compressed, the structure of the tissue allows it to yield slightly and release synovial fluid, which provides superior lubrication, lessens the impact of loads and, as consequence, prevents wear. Prostheses on the market fall into one of two categories, none of which correspond to the native case: hard-on-soft and hard-on-hard.

Hydrogels have gained attention in this field due to being biocompatible and their ability to retain a high water content. Of these, PVA hydrogels has become an attractive material for cartilage replacement applications. PVA is biocompatible and good swelling properties.[4] The characteristics of the resulting hydrogel may also be tailored by adjusting the production method[5,6] or by combining PVA with other materials to produce a more suitable and stable material.[7–9] Furthermore, there are already some clinical applications of PVA, such as surgical sponges, contact lenses and hydrophilic coating for catheters.[4]

The objective of this work is to create a protocol for the production of (nine) PVA-based hydrogels as potential cartilage replacement for the rubbing surface of TKA or THA and perform their characterization. Namely, all manufactured gels have been characterized regarding their swelling behaviour, wettability, thermotropic behaviour and mechanical properties.

2. Materials and Methods

2.1. Preparation of PVA hydrogels

A total of nine different PVA hydrogels (PVA-H) were fabricated by cast drying using three different weight PVA powders and other compounds (none, PVP, PVP and glyoxal). Low and high molecular weight PVA powders (PVA L and PVA H, respectively) were obtained from Sigma Aldrich, medium molecular weight PVA (PVA M) was obtained from Kuraray, PVP-K30 was obtained from BASF SE and 40% w/w aqueous solution of glyoxal was purchased from Alfa Aesar. The nature of the experiments demanded the production of two different thicknesses of gels (aproximately 0.5mm for thin gels and 1.5mm for thick gels).

The gels were prepared with an initial concentration of PVA of 15 wt% following data collected from previous

literature [7,10–13]. Nine different solutions were prepared where PVP was used at a concentration of 1%[7,12,14] of the solid solutes, and glyoxal at 0.02%[15].
10 ml (for thin gels) or 75 ml (for thick gels) of PVA solutions were prepared by dissolving the solute in DD water in an oven at 95ºC for 24 hours. At the intermediate time10-15hr the solutions were stirred using the vortex. An additional step was performed whenever air bubbles were still present in the solution after the 24hr period – the solution was subjected to sonication inside a 95ºC water bath to keep viscosity at a minimum and allow the release of air bubbles. The solutions were then cast into glass molds (e.g. petri dishes) and placed in a 37ºC oven with limited air circulation.
Finally, the gels were washed. The samples were submitted to 24h washing cycles using DD water, until dissolved substances were no longer detected. The water was renewed and analyzed in a Multiskan GO 1.00.40 spectrophotometer after each cycle (start wavelength: 200nm; end wavelength: 700nm. Samples were considered clean at absorbance values below 0.1.

2.2. Samples characterization

2.2.1. Swelling Behaviour

The test conditions to measure the swelling ratio were thus defined as follows: Pre-washed, hydrated gels were cut into 10mmx5mm strips and dried at 36ºC until the mass stabilized. The dry samples were then placed inside 15ml lab falcons with 2ml of DD water each, shielded from the light. The PVA-H strips were kept at 36ºC, a temperature close to that of the human body, and the mass was measured throughout a day until a peak was reached – typically four measurements over the course of 3 hours were sufficient -, and then a week later. The excess moisture in each sample was carefully removed before each weighing. At least 3 repetitions were performed for each formulation.
The percentual swelling ratio, %SR, is defined as follows:

$$\%SR = \frac{w_h - w_d}{w_d} \times 100 \qquad (1)$$

Where w_d represents the weight of the dried sample and w_h represents the weight of the hydrated sample.
The equilibrium water content, %EWC, is defined as follows:

$$\%EWC = \frac{w_h - w_d}{w_h} * 100 \qquad (2)$$

Where w_d represents the weight of the dried sample and w_h represents the weight of the hydrated sample.

2.2.2. Wettability

For the sake of simplicity, an approximation to an ideal surface was considered and the principles of the Young-Dupré Equation have been applied. The wetting properties of the hydrated gel were determined by calculating the contact angle (θ) at the three-phase contact point between the tangent to the contour of a captive bubble (air) and the surface of the sample immersed in DD water.

To carry out the captive bubble procedure, a goniometer was used - A JAI CV-A50 camera, connected to a Data Translation DT3155) frame grabber and supported by a Wild M3Z optical microscope. During 5 minutes, 17 images of a single bubble were acquired. The value of the contact angle was measured on picture 17, when the bubble was stable. 7 to 10 consistent bubbles were done for each hydrogel.

2.2.3. Thermotropic behaviour

Differential Scanning Calorimetry (DSC) was used to study the thermal transitions of polymers, such as melting temperature or glass transition. A Netzsch DSC 200 F3 Maia machine was used for this purpose. The equipment was controlled with the DSC 200F3 software and the results were analysed on Proteus Analysis.
The technique was performed in two ways, depending of the state of hydration of the samples. The hydrated samples were subjected to a full heating and cooling cycle, ranging from -35ºC to 40ºC at a rate of 10ºC/min. In this test, the intention was to observe the amounts of free and loosely bound water in the samples.
The test was also performed on dry samples. In the literature, Tg of PVA is between 85-88ºC [16,17,8], the Tm is at approximately 210-219ºC [16,8,17] and thermal degradation occurs as a two-step degradation at 300-450ºC and 450-550ºC [8,18]. Based on this information, the dry samples went through one cooling cycle, one heating cycle and one cooling cycle again, from 20ºC to 250ºC at a rate of 10ºC/min. The goal was to study the effect of the composition on the thermotropic behaviour of the gels, namely variations in glass transition temperature enthalpy of phase transition.
For the dry test, the samples were previously cut into small pieces, dried at 36ºC for 5 days with ventilation and then for an extra 5 days at 36ºC with vacuum, totalizing 10 days of total drying time. Samples weighed approximately 3mg each. The equipment is very sensitive to water, rendering inaccurate results if the samples were not conveniently dried. Even at a small size, these gel's ability to retain water is extremely high which is why vacuum became necessary. After drying them thoroughly, the samples were kept in a desiccator until use.
The degree of crystallinity was calculated as follows:

$$Crystallinity\ (\%) = \frac{\Delta H_f}{\Delta H_{f*}} * 100 \qquad (4)$$

Where ΔH_f is the specific energy/enthalpy of the sample, and ΔH_{f*} is the enthalpy of pure PVA (138.6 J/g).[19]
The percentage of free water in the samples was calculated as follows:

$$\%\ Free\ and\ loosely\ bound\ water = \frac{\frac{\Delta H_f * w_h}{\Delta H_w}}{\frac{\%EWC * w_h}{100}} \qquad (5)$$

Where ΔH_f is the specific energy of the sample, w_h is the weight of the hydrated sample, $\%EWC$ is the percentual equilibrium water content and ΔH_w is the enthalpy of water (334 J/g)[20]

All tests were performed in at least duplicate.

2.3. Mechanical Properties

By plotting the stress against the strain, the characteristic stress-strain curve of the material (called the engineering curve) can be obtained.
The Young's modulus or modulus of elasticity (E) is the proportionality constant between the stress and strain in the linear region, and serves as a measure of stiffness of the material. It depends on the interatomic forces: the stronger the bond, the higher the value of E is and thus, the higher the stiffness. This property can be calculated using Hooke's Law:

$$E = \frac{\sigma}{\varepsilon} \qquad (8)$$

where E is the Young's modulus, σ is the stress and ε is the strain, as referred above. The Young's modulus was extrapolated from tension and compression stress-strain curves.
The ultimate tensile strength (UTS) is the maximum stress that a material can withstand while subjected to a tensile force. Toughness is the energy absorbed by a material before fracture. It can be calculated by integrating the stress-strain curve from the beginning of the test to the moment of rupture.
Tensile tests were performed on a TA.XT Express Texture Analyser with miniature tensile (serrated) grips by applying force to the test samples. The most reproducible results were obtained for flat tensile specimens, cut out of the thicker gels using a custom-made punch. (Dimensions: a gage length of 8mm and 2mm width, a distance of 15mm between shoulders and an 8.5 mm grip section.)
Miniature tensile grips were attached to a TA.XT express texturometer. The grips were set to a calibration height of 15mm and a maximum displacement of 80mm, at a constant strain rate (1mm/sec). Additionally, sandpaper was glued to the grips, to prevent slippage of the hydrated gels. Stress-strain curves were then obtained, the Young's Modulus was extrapolated from the linear/elastic region of the curves and the ultimate tensile stress and maximum strain rate were measured, as well as the material toughness. A minimum of 3 repetitions per sample were done.
Unconfined compression tests were performed on 8mm diameter circular PVA-H samples. The test was conducted in a homemade equipment with customized software on LabViewer. To achieve unconfined compression, an indentation attachment was placed on the machine and a small, 10mm diameter circular titanium piece was placed on top of each of the samples. 25N of force were applied to the hydrated hydrogels and the resulting data were analyzed to extrapolate the Young's Modulus and compressive toughness. At least 4 specimens of each sample were tested.

3. Results and Discussion

3.1. Perfecting the Protocol

Two different versions of FT (freeze-thawing) protocols were tested, but none were successful. The first attempt consisted of depositing the pre-gel solution between two pieces of sylanized glass. 3 FT cycles (1hr -80ºC, 1hr room temp.) were performed. There was not enough oxygenation and gelation did not occur.
The second FT protocol included additional and longer FT cycles (16h -20ºC, 8h room temp.). The resulting gels were translucent and they partially dissolved during the swelling test.

Figure 1: Partially desintegrated FT gel.

Gels were also produced by cast-drying (CD), and these were the gels that were ultimately used for this work. An initial attempt rendered a very flat, transparent, homogeneous hydrogel, perfect for the testing purposes. This gel was obtained by very slow gelation that involved a water pump that achieved a weak vacuum seal in a desiccator. This method was wasteful and not scalable for bigger gels so the protocol had to be changed.
Obstacles to overcome included striking a balance between oxygenation and drying temperature. Low aeration and rapid cooling induce striations (Fig.2) but vacuum causes air bubbles to be trapped within the gel (Fig.3). On thick gels, semi irreversible contracture occurred (Fig.4)

Figure 2: Striations of the gel upon rapid cooling and oxygen exposure of the pre-gel solution.

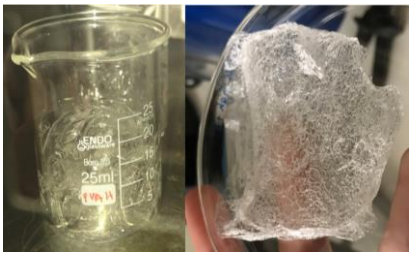

Figure 3: Extreme removal of oxygen: vacuum made water come to a boil and air bubbles solidified onto the gel.

Figure 4: Semi irreversible contracture of thick PVA hydrogels.

Finally, with the protocol described in section 2.1., it was possible to achieve flat, fairly homogeneous and transparent for both thin and thick gels.(Fig 5. and 6.)

Figure 5: Thin PVA hydrogels: up and side views.

Figure 6: Thick PVA hydrogel.

3.2. Samples characterization

3.2.1. Physical Properties

3.2.1.1. Swelling Behaviour

Swelling is extremely fast in all samples, denoting the high hydrophilicity of PVA and PVP.[21] In 30 minutes, equilibrium swelling is almost reached. In all %SR profiles except for pure PVA samples, the swelling ratio peaks at the 30-minute mark, and stabilizes at a slightly lower value over the course of a week. Initially, water penetrates the samples and the gel network expands. This induces the rearrangement of polymer chains, stimulating the formation of new hydrogen bonds between PVA molecules. The balance between the rates of osmotic diffusion and rearrangement of the chains is a possible explanation for this overshooting phenomenon.[22]

A typical %SR profile for a PVA sample of this kind can be found in Fig. 7. The data analysis of %SR and %EWC can be found in Table 1.

Swelling rates vary between 138% and 308% across the board. In terms of water content, the water content is progressively lower as molecular weight increases. This is consistent with the concept that heavier chains form a tighter network of bonds, thus leaving less space in the amorphous region of the gel for water to fill.[23]

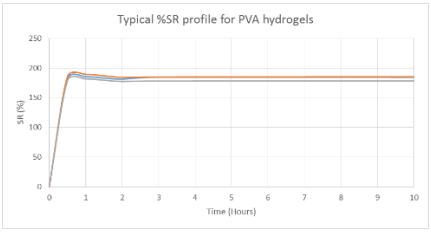

Figure 7: Typical ST% profile of a PVA hydrogel displaying an overshooting phenomenon at the 30-minute mark.

Table 1: Average Swelling Ratio (%) and Equilibrium Water Content (%) for the tested gels.

Material	**%SR**	**%EWC**
Native Cartilage	-	65 - 85
PVA Hydrogels in the literature	CD & FT: 260 - 320[23,24]	61 – 70[23,25]
PVA L	195 ± 2	66.1 ± 0.3
PVA L + PVP	238 ± 19	70.4 ± 1.7
PVA L + PVP + G	308 ± 14	75.4 ± 1.5
PVA M	163 ± 2	62.9 ± 0.7
PVA M + PVP	166 ± 11	62.4 ± 1.5
PVA M + PVP + G	184 ± 6	64.8 ± 0.7
PVA H	138 ± 3	58.0 ± 0.6
PVA H + PVP	149 ± 6	59.8 ± 1.0
PVA H + PVP + G	152 ± 8	60.2 ± 1.3

Cartilage is approximately 65% to 85% water[26], All gels seem to be within or very close to the %EWC range found in the human articular cartilage. Moreover, the values obtained are in accordance with values from the literature, including recent research that compares the swelling properties of PVA/PVP hydrogels with the exact same polymer proportions, which showed results for the EWC in a similar range (61% - 68%).[23] These findings are thus not only consistent with comparable work by other authors, but also with the water content in the deep zone (65%), which is responsible for most of the mechanical resistance of cartilage.[27]

Generally speaking, however, it would appear that PVP causes the swelling capacity of the samples to increase. This is possibly due to the chain rearrangements that take place when PVA and PVP crosslink – bulky pyrrolidone rings interrupt PVA crystalline chains and form larger pores.[21,28] It has also been hypothesized that the high affinity to water in amide groups is a possible explanation to the increased swelling capacity in PVP hydrogels.[7]

Oddly, the presence of glyoxal seemed to have little to no effect on the swelling behaviour of the material. It was hypothesized that perhaps chemical crosslinking did not occur. For this reason, there was an attempt to solubilize the hydrogels at two different temperatures. The gels dissolved and became an aqueous solution again when heated at 95ºC, but they were stable

stable up to 50ºC, covering the range of temperatures compatible with human life.
The importance of swelling behaviour stems from its connection to the mechanical and tribological properties of the hydrogel, as well as how it impacts the risk of implant failure. In 2007, PVA hydrogels were used for treatment of knee cartilage defects in adult rabbits. Results revealed growth over the implant and implant shrinkage.[30] Gels can react to osmotic gradients and swell and de-swell accordingly, even in hydrated conditions. This volume change may induce detachment from the tissue or implant and interfacial debonding. For this reason, this is perhaps one of the most important aspects to consider when measuring the swelling properties. In this sense, a lower swelling capacity would be desirable, since it would provide a greater stabilization in the contact surface or attachment between the PVA implants and bone or metallic alloys.

3.2.1.2. WETTABILITY

The contact angle obtained for the produced PVA hydrogels is generally low, which indicates high hydrophilicity. The swelling behaviour of these samples, which swell up to 3 times their dry weight, is in line with these results. On average, the contact angle decreases, and thus wettability increases, with the increase of the molecular weight of PVA. A possible explanation would be that the length of the heavier PVA chains may be a limitation in the assembly of tightly packed chains upon gelation, thus forming larger amorphous regions.
It can also be observed that, the addition of PVP and glyoxal increases the wettability of the samples. The concepts that are believed to explain the swelling behaviour of the samples may also be employed for the wetting properties of the material: bulky pyrrolidone rings may prevent the formation of tigher bonds within the PVA and form large pores[21,28], and the high affinity between water and amide groups in PVP may further contribute to the hydrophilicity of the gel.[7]
Glyoxal made the samples more hydrophilic, the exception being PVA H + PVP + G. The decrease of the contact angle with the increase of crosslinking agents has been reported before.[31] It is possible that the addition of glyoxal hardened localized clusters of high bonding density, giving rise to inhomogeneity in the polymer surface. This interfered with the cohesive bridging flocculation of the polymer, decreasing the contact angle.[32]

Table 2: Summary and comparison of measured contact angle in PVA-H VS the literature.[33–36]

Tissue	**Contact angle (º)**	**Method**
Normal human AC	94 - 105	Sessile Drop
Normal bovine patella	100	
Human knee	80	
Arthritic Knee	63	
Human hip	76	
Arthritic hip	56	
PVA in the literature	37 – 45 [37,38]	Captive Bubble
PVA L	51 ± 6	Captive Bubble
PVA L + PVP	50 ± 4	
PVA L + PVP + G	39 ± 5	
PVA M	48 ± 8	
PVA M + PVP	38 ± 6	
PVA M + PVP + G	33 ± 3	
PVA H	45 ± 3	
PVA H + PVP	31 ± 4	
PVA H + PVP + G	32 ± 4	

The measured angles are drastically different from those seen in the literature for natural cartilage, only coming close to the values observed for osteoarthritic joints (Table 2). One important factor to note, however, is that all the data from the literature was obtained by means of the sessile drop method, as opposed to this work, where the captive bubble method was used.
Wetting is not a static state – there are numerous stable metastates of a droplet of water on a surface. The sessile drop method is analogous to the maximum end of the spectra, called the advancing contact angle, whilst the captive bubble measures the receding contact angle, at the lower end of the range. Contact angle hysteresis thus becomes a possibility. According to the literature, contact angle hysteresis is a consequence of heterogeneity and surface roughness.[39–43] Young's equation considers and ideal, perfectly flat solids, where hysteresis does not exist. In reality, most surfaces present surface irregularities that act as barriers to the motion of the contact line, which can alter the macroscopic measurement of contact angles.
No reference values for the captive bubble method have been reported for the natural cartilage. However, compared to other PVA constructs, the range of values obtained overlaps with those observed in previous research.

3.2.2. MECHANICAL PROPERTIES

A homemade customized punch was made in order to produce specimens with a large contact area between the serrated grips and the gel in order to have a better distribution of forces. The goal was to fabricate specimens where tensile forces would focus along a rectangular length and induce rupture in the middle. This technique was rather successful: most gels ruptured along the gage length of the specimen.
The protocol for the compression tests, as defined in Section 3.3, was effective and the resulting hydrogels rendered consistent results as well.
From the data obtained, stress-strain curves were plotted, the tensile and compressive YM were calculated, and the UTS, the maximum strain rate, the fracture toughness, and the compression strength were obtained. An overview of the typical stress-strain curves of all the formulations of PVA-H can be seen in Fig. 8 and 9.

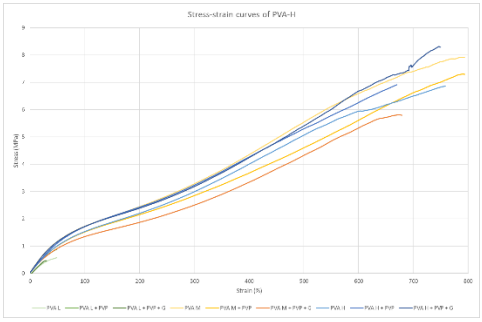

Figure 8: Illustrative example of the tensile stress-strain curves of PVA-H.

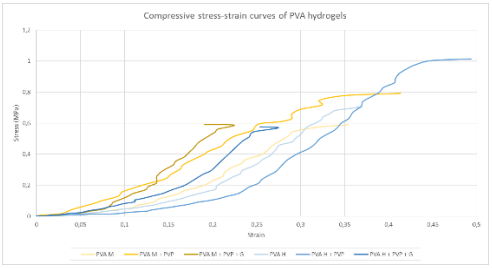

Figure 9:Illustrative example of compressive stress-strain curves of PVA-H.

The YM was obtained by adding a linear trendline in the elastic portion of the stress-strain curve of both tensile and compressive tests and taking the slope of its equation (which is the YM).
The UTS and the maximum strain rate were obtained by direct measurement though the stress-strain curves. The UTS and the maximum strain rates are is the maximum stress the samples withstand, or the maximum strain observed, without breaking.
The fracture toughness and the compressive strength are the areas under the stress-strain curves.
Some of the values obtained are an estimation, since some test specimens did not break or slipped.
The UTS of PVA L is at the lower end of the range of the UTS in the natural cartilage, and the fracture toughness is significantly lower than articular cartilage and other PVA-H formulations. Because of its weak mechanical properties, further analysis of PVA L was not pursued.
Table 3 summarized all measured properties and comparison with the literature.

Table 3: Summary of the mechanical properties of natural cartilage, PVA in the literature and PVA in the scope of this work.

Material	**Tensile YM (MPa)**	**UTS (MPa)**	**Toughness (MPa/mm$^{0.5}$)**	**Compressive YM (MPa)**
Native cartilage	4.3 – 25[49]	0.8 – 25[49]	305 - 391[50]	0.24 – 1[49]
PVA in the literature	0.19[5] 1 1.4[52]			0.07 – 0.24[53] 2.56 – 3.68[4]
PVA L	2.1 ± 0.3	0.7	18 ± 3	-
PVA L + PVP	2.3 ± 0.2	0.9	34 ± 6	-
PVA L + PVP + G	1.8 ± 0.2	0.5	11 ± 1	-
PVA M	2.4 ± 0.3	≥ 8	≥ 493 ± 26	3.6 ± 0.4
PVA M + PVP	2.5 ± 0.2	≥ 7	≥ 529 ± 37	3.1 ± 0.5
PVA M + PVP + G	2.0 ± 0.2	≥ 6	≥ 458 ± 22	4.1 ± 0.5
PVA H	2.4 ± 0.4	≥ 7	≥ 543 ± 7	4.5 ± 0.5
PVA H + PVP	2.6 ± 0.3	≥ 11	≥ 574 ± 17	3.4 ± 0.6
PVA H + PVP +G	2.7 ± 0.3	≥ 8	≥ 592 ± 12	4.1 ± 0.3
Native cartilage	4.3 – 25	0.8 – 25	305 - 391	0.24 – 1

For the remaining PVA M and PVA H hydrogels, the UTS values presented are well within the limits of articular cartilage, which can go up to 25 MPa. Due to slippage, minor misalignments that induce premature breaking or anisotropies in the material, it is plausible to conclude the data obtained does not reflect the apex of the capabilities of the material. The highest UTS was measured in PVA H + PVP (10.77 MPa) which, in actuality, did not rupture (Fig 10). As a result, there is strong evidence that suggests that, under certain circumstances, this material could withstand even higher loads.
The tensile YM was low both in tension and compression. A low YM denotes a flexible material that deforms easily under loads.
In theory, tensile and compressive elastic moduli should be equal, which is not observed in the case of cartilage and PVA constructs, both self-made and referenced material. The calculated tensile and compressive YM are slightly different in the case of the tested hydrogels The YM is, in reality, dynamic, and depends on test conditions such as time, temperature, strain rate and fiber orientation.
Anisotropy is one of the main reasons why PVA might behave different to tensile and compressive stresses and have dissimilar YM. The results here presented support the idea that PVA is an anisotropic material, as well as cartilage.
The YM and toughness show that natural cartilage is tougher when subjected to tensile loads and softer when compressive loads are applied (Table 4). Presumably, it is this elastic behaviour upon compression that allows the release of synovial fluid and enables efficient lubrication. In this sense, it is not advantageous that PVA-H seem to be stiffer under compressive loads.

Table 4: Toughness properties under tension and compression for PVA-H at a stress level of 500kPa.

	Fracture Toughness (MPa/mm$^{0.5}$)	**Compressive Toughness (MPa/mm$^{0.5}$)**
PVA M	≥ 5.7 ± 0.4	≥ 48 ± 4
PVA M + PVP	≥ 5.2 ± 0.4	≥ 51 ± 4

PVA M + PVP + G	≥ 6.4 ± 0.1	≥ 43 ± 8
PVA H	≥ 5.6 ± 0.8	≥ 40 ± 4
PVA H + PVP	≥ 5.2 ± 0.1	≥ 52 ± 10
PVA H + PVP +G	≥ 4.5 ± 0.2	≥ 43 ± 4

According to the curve in Fig 10, PVA H + PVP displays a great capacity to deform under loads, reaching strain levels of approximately 8.2. After unloading, it can be observed that the hydrogel recovers partially by 4.7 (elastic deformation) but plastic deformation also occurs, since the end specimen is 3.5 times its original size.

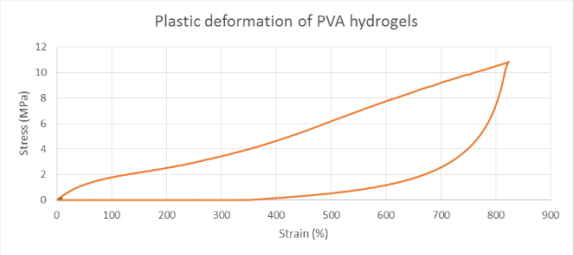

Figure 10: Stress-strain curve showing plastic deformation upon unloading.

3.2.3. Thermotropic Behaviour

The thermotropic behaviour of the PVA hydrogels was documented and compared with the literature. Fig. 11 contains all the data concerning DSC of dry samples, and Table 5 summarizes all the properties extrapolated through this test. It is important to note that these tests were initiated after mechanical testing, at which point the tensile data had already revealed PVA L was not the most fitting material for cartilage replacement applications. As so, at the time of DSC, the decision not to further pursue PVA L studies had already been made, and this section features only PVA M and PVA H hydrogels.

The Tm is an important measurement of the degree of purity of the substance. According to the literature, PVA melts at approximately 210 – 215ºC[19]. Tubbs[44] investigated the influence of heating rate on the Tm of PVA and concluded that for a 10ºC/min heating rate (used in this work), the Tm is 225.8ºC. The Tm of the hydrogels was very similar to the values found in the literature - around 227-228 ºC for PVA M and 229-230ºC for PVA H.

The degree of crystallinity varies from 43% to 49%. This indicated a semi-crystalline material with both crystalline and amorphous regions. The presence of amorphous areas originates disorder, reducing the enthalpy of the system.

Table 5: Thermotropic behaviour of the PVA hydrogels under dry conditions.

Dry samples	**Tm (ºC)**	**Specific Energy (J/g)**	**Degree of Crystallinity (%)**
PVA M	227.8 ± 1.0	61.5 ± 3.7	44
PVA M + PVP	227.5 ± 0.9	60.1 ± 0.6	43
PVA M + PVP + G	228.3 ± 0.2	64.6 ± 0.6	47
PVA H	229.8 ± 0.4	67.4 ± 4.5	49
PVA H + PVP	230.9 ± 2.0	68.4 ± 0.5	49
PVA H + PVP + G	230.1 ± 1.2	66.3 ± 2.1	48
PVA in the literature	210 – 215 [19,46,47]	-	45 – 46[23]

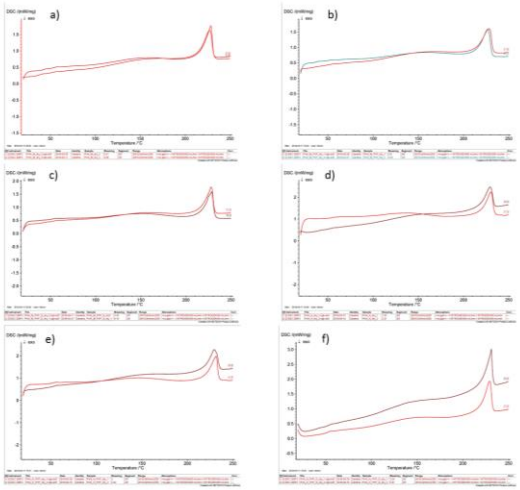

Figure 11: DSC curves of dry samples. a) PVA M; b) PVA M + PVP; c) PVA M + PVP + G; d) PVA H; e) PVA H + PVP; f) PVA H + PVP + G.

The Tg of PVA is usually found at around 85ºC[45] but could not be identified for any of the samples. The Tg is typically more difficult to locate, especially when the Tm is very pronounced, because it can occur over a very small range of temperatures that make it extremely difficult to identify. On the other hand, glass transition is associated with segmental motility of the amorphous region, which could be impaired due to high density of hydrogen bonds and crosslinking. Therefore, it is also entirely possible for a polymer not to exhibit a Tg. However, the degree of crystallinity shows that the amorphous region exists, and it is consistent with the fact that PVA is a semi-crystalline polymer. As so, the hypothesis that a Tg exists but could not be detected is more likely.

To evaluate the effect of the presence of free, loosely bound and tightly bound water in the samples, the tests were also conducted in the hydrated state (Table 6).

According to the calculations, the percentage of free and loosely bound water is about 74% for PVA M and 65% for PVA H – these values qualitatively align with those obtained for the swelling behaviour.

In Fig. 12, in most images, two overlapping peaks are distinguishable. The first, occurs at negative temperatures, while the second occurs at around 0ºC. The first peak corresponds to loosely bound water and the second, to free water. Only the higher peak was measured, which in most cases corresponds to a

slightly negative temperature, and should coincide with loosely bound water. Thus, from the images and the measured peak temperatures, it is apparent that within the free and loosely bound water percentage, a higher amount of that water is loosely bound water on the hydrogels. This may indicate that, despite the high degree of swelling and convenient water content, it is possible that a lot of this water stays within the matrix when cartilage is submitted to pressures and less water is available to lubricate the surface. The hydrophilicity observed in the gels, even with regards to the literature, supports the theory that most water is retained within the polymer matrix.

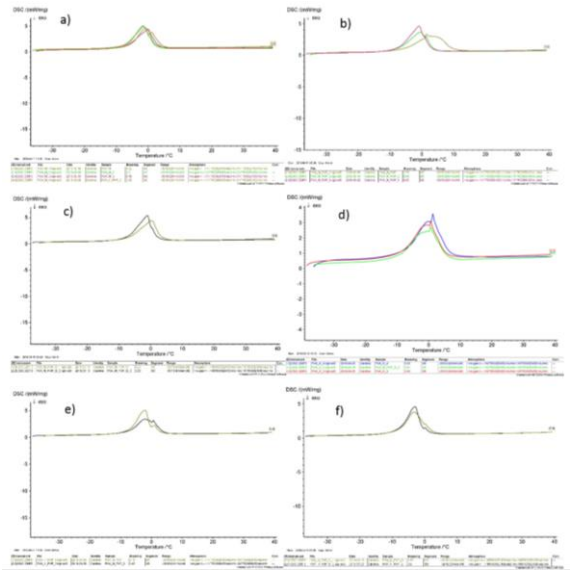

Figure 12: DSC curves of hydrated samples. a) PVA M; b) PVA M + PVP; c) PVA M + PVP + G; d) PVA H; e) PVA H + PVP; f) PVA H + PVP + G

Table 6: Thermotropic behaviour of the PVA hydrogels under hydrated conditions.

Hydrated samples	**Tm (ºC)**	**Specific Energy (J/g)**	**Free and Loosely Bound Water (%)**
PVA M	-0.6 ± 0.9	156 ± 6	75 ± 3
PVA M + PVP	0.6 ± 1.0	153 ± 9	73 ± 4
PVA M + PVP + G	-0.4 ± 0.9	159 ± 6	74 ± 3
PVA H	0.9 ± 0.5	127 ± 7	65 ± 4
PVA H + PVP	-2.1 ± 0.0	146 ± 14	73 ± 7
PVA H + PVP + G	-3.2 ± 0.2	134 ± 0	67 ± 0

4. CONCLUSIONS

After several iterations of the protocol, it was possible to create a simple protocol for the production of physically crosslinked, CD PVA hydrogels. The end product consisted of transparent, flat, homogeneous physically crosslinked gels. While the process is not seamlessly upscaled to produce thicker hydrogels, the results were very consistent.

All the different PVA hydrogels that have been studied present a significantly larger water content percentage in their swelled state, with swelling rates varying between 138% and 308% across the board. Regarding their %EWC, all gels seem similar to natural cartilage and other PVA constructs. Generally speaking, all PVA hydrogels swell significantly in the presence of water. %SR and %EWC both increase when the molecular weight of the PVA decreases. This is consistent with the concept that heavier chains form a tighter network of bonds, thus leaving less space for water to fill.

%SR and %EWC also increase as PVP and glyoxal are added to the solution. This might be due to bulky pyrrolidone rings which prevent the formation of tighter bonds and the high affinity to water of PVP.

Glyoxal seems to have little to no effect on the gels.

All PVA hydrogels tested exhibit values that are not far or within the range of natural cartilage, with %EWC ranging from 60% to 75%.

The samples were found to be very hydrophilic, with low contact angles in the interval 32º - 50º. The trends observed are in line with those of the swelling behaviour – the contact angle decreases with 1) the increase of molecular weight; 2) the addition of PVP and glyoxal. Similar values have been reported in the literature for the captive bubble method (37º - 45º) [260,261]

Here, a static contact angle has been considered, but the differences in the sessile drop method and the captive bubble test also demonstrate that contact angle hysteresis exists. It is believed that contact angle hysteresis is a consequence of heterogeneity and surface roughness. The existence of surface roughness on PVA hydrogels has also been confirmed by the literature.

As far as the mechanical properties go, the tensile and compressive YM were calculated, and the UTS, the maximum strain rate, the fracture toughness, and the compressive strength were obtained.

The first conclusion that can be drawn from the stress-strain curves and its derivative properties is that PVA L is rather below the desirable range of values for UTS or fracture toughness. Its UTS sits on the lower limit of the registered values of articular cartilage and the fracture toughness of cartilage is at least 10 times larger than that of PVA L. Very early on into the mechanical testing, PVA L displayed weak mechanical properties and for that reason it was put aside.

The fracture toughness of PVA M and PVA H is already greater than that of the cartilage at just 300% elongation. Some gels have been seen to reach over 800% elongation, which means that the PVA-H hydrogels are, in this instance, superior to cartilage.

UTS values for PVA M and PVA H hydrogels are encompassed in the interval determined for the articular cartilage. The UTS in the literature for the articular cartilage ranges between 0.8 and 25 MPa.[79] The tested PVA-H go from 5.7 to 10.8 MPa. Once again, these measurements should, however, be but an underestimation of the true value, since the anisotropies of the material can sometimes lead to premature failure and, in some cases, slippage of the test specimens occurred.

One of the hydrogels, PVA H + PVP, reached the highest measured UTS value across the board (10.8 MPa) without rupturing. According to this curve, PVA H + PVP displays a great capacity to deform under loads, reaching strain levels of approximately 8.2. Part of this deformation was seen to be elastic (4.7), while a smaller portion of it was plastic (3.5). Overall, this indicates that, under certain circumstances, this material has the capability to go beyond these values.

The anisotropies of the gels are confirmed upon the knowledge that the YM differs in tensile and compressive conditions. Toughness at 500 kPa for both tensile and compressive tests further supports this hypothesis. In fact, from the YM and the toughness of the material in tension and compression, one can conclude that the PVA-H samples are stiffer under compressive loads than under tensile loads. Whereas this trend is in accordance with PVA-H in the literature, it is opposite to the trend seen in articular cartilage.

As for the thermotropic behaviour of PVA, the dry tests should allow for the extrapolation of the Tg, the Tm and the degree of crystallinity. The results regarding the Tm were very comparable to those found in the literature: all the tested PVA hydrogels presented values within the 227-228ºC range. The degree of crystallinity varies from 43% to 49%. This is consistent with PVA being a semi-crystalline material. The Tg of PVA could not be identified for any of the samples.

The gels contain about 66% (PVA H) to 74% (PVA M) of free and loosely bound water. Analysis of the DSC curve suggests that most of the water in the gels is loosely bound water.

As far as swelling behaviour goes, all gels were within or very close to the values observed in cartilage. From this parameter, all materials are adequate or acceptable. In what concerns wettability, no comparable results exist for the cartilage. However, it is known to be highly hydrophilic. The mechanical testing rapidly excluded PVA L as a good candidate for cartilage replacement because of inadequate toughness. All the other gels performed favourably and may be potentially used. As for the thermotropic behaviour, the results were remarkably similar to the reference material both for PVA M and PVA H. All in all, the best materials resulting from this work, according to the data, are PVA M + PVP + G, followed by PVA M, PVA M + PVP, PVA H, PVA H + PVP and PVA H + PVP + G.

5. Future work

The tribological properties could not be ascertained during the course of this work, due to lack of time. It would be important to complete the data in this thesis with information regarding the coefficient of friction. Water is thought to underestimate the coefficient of friction, so the use of other lubricants, such as hyaluronic acid (present in the synovial fluid), could be advantageous.

Also, the relationship between wettability, surface morphology and coefficient of friction should be studied, since information in the literature suggests these properties influence the tribological behaviour of the samples.

The study of the cell adhesion properties of PVA-H could be interesting for applications in the replacement of localized defects of the cartilage. While this is not the application initially planned, many studies place importance on cell adhesion for appropriate integration of hydrogels in partially injured cartilage.

Moving forward, it might be interesting to explore other gelation methods. Namely, freeze-thawing might be a compelling course of work, since its properties can be tailored by changing the number FT cycles.

Still regarding alternative gelation processes, ionizing radiation is another pertinent suggestion. PVA must be sterilized for this type of biomedical applications. Methods that resort to heat are not adequate for CD PVA hydrogels, for example, since the heat compromises the integrity of the gel. Ionizing radiation is the most frequently reported sterilization method for hydrogels, along with autoclaving. However, ionizing radiation induces gelation and sterilizes in one step. Surely, introducing gamma rays as a variable in the protocol could generate very interesting results.

Furthermore, the environment surrounding these gels in their intended application would be very fragile as a result of inflammation. Following up on the possibility of embedding the polymer matrix with therapeutic drugs and achieving their controlled release would be key.

Lastly, extending this study to other materials, such as alginate, chitosan, PEG and others might be an asset.

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