Tribomechanical behaviour of chemically crosslinked PVA hydrogels

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Abstract

Poly (vinyl alcohol) (PVA) hydrogels have been considered promising materials for articular cartilage replacement. The most vital shortcomings of PVA hydrogels is their ability to mimic the demanding mechanical and tribological properties of articular cartilage.

In the present work, PVA hydrogels crosslinked with different amounts of glyoxal and glutaraldehyde were prepared by cast drying, using two different PVA molecular weight. The best performing hydrogels were loaded with an anti-inflammatory, diclofenac. Most of the hydrogels had internal three-dimensional structure and water content (57%-80%) close to natural articular cartilage. Glyoxal in concentrations of 0.2% wt and 1% wt slightly improved mechanical and tribological properties, increased elastic modulus, stiffness and lowed the coefficient of friction. Glutaraldehyde in concentrations of 0.75% wt and 1.5% wt, in general, led to the same conclusions but with a wider variety of results, whereas at concentrations of 3% wt,6% wt and 12% wt the hydrogels did not had the adequate mechanical properties. It has also been observed that, tribologically and mechanically, higher molecular weight hydrogels behave similarly to natural cartilages, with higher tensile than compressive strength. Nevertheless under tensile forces the hydrogels' strength remains lower than cartilage.

The coefficients of friction were low, for all samples, ranging between 0.047-0.07.

The PVA hydrogel with higher molecular weight and 1 wt% glyoxal had the best mechanical properties and although the coefficient of friction of such hydrogel is comparable with natural cartilage and other PVA hydrogels described in literature, its resistance to friction remained low. Lastly, the anti-inflammatory release from the hydrogels occurred within the first 8 hours. The addition of the crosslinkers did not significantly affected the drug release profile , whereas the increase in PVA's molecular weight slightly decreased the quantity of drug released.

Keywords: PVA hydrogels, chemical crosslinking, articular cartilage replacement, mechanical properties, tribological properties

1. Introduction

The joints are the largest load bearing biological friction pairs. Therefore, in healthy natural synovial joints, articular cartilage (AC) covering the joint surface, bears and distributes loads caused by the different movements from daily activities, during the whole life. Providing stability and superior lubrication mechanism, maintaining low friction and minimum wear [1, 2, 3], AC is responsible for cushioning compressive forces in the joint.

Deterioration and wear of cartilage leads to symptoms like joint pain, stiffness, swelling and impaired mobility [4, 5], finally leading to joint diseases, like osteoarthritis (OA), which has become highly prevalent around the world [6]. In fact, about 400 million people suffer the joint disease, and with life expectancy continuing to increase, this has become a big problem. Arthritis disables and restricts daily activities causing serious pain in severe cases [7].

AC has limited healing potential due to its avascular and aneural nature [8]. Replacement of joint, is an effective surgical treatment to relieve pain and to restore full mobility to patients with damaged joints [9]. Most artificial total joint replacement materials are rigid materials such as metals, ceramics, and ultra-high molecular weight polyethylene (UHMWPE), whose stiffness is much higher compared to natural AC. Besides the compatibility with the human medium is poor. Moreover, the contact interface of current artificial joint implants is hard-on-hard, which would cause excessive wear and absence of lubrication [6]. The major reasons of failure of the artificial joint implants are osteolysis and aseptic loosening due to wear, which represents a serious concern for young and active pa-

tients, due to the limited lifespan of the implants.

Poly(vinyl alcohol)(PVA) hydrogel has emerged as an alternative for articular cartilage substitution. It has a three-dimensional network structure, which is similar to natural AC, with high swelling capacity and great compliance, whose main component is water [4]. Meanwhile, it also has good biocompatibility, and, depending on the preparation conditions, may present suitable tribological performance and load-bearing properties [10]. When submitted to load, PVA hydrogel release water from its network. providing superior lubrication [9]. Being a biphasic material (solid phase + water phase), PVA hydrogels may be a closer mimic to cartilage by enabling cartilage-like viscoelasticity and biphasic lubrication [11, 12] with a low coefficient of friction in the range of 0.02 to 0.07 against smooth and wet substances [4, 13].

Chemical crosslinking may be used to improve PVA hydrogels properties, such as mechanical and chemical stability [14]. Common crosslinkers used are dialdehydes such as glutaraldehyde and glyoxal.

Glyoxal is biocompatible and non-toxic [15], reacts with the hydroxil groups from PVA to form covalent acetal bonds between PVA chains, improving the mechanical strength of the gel [16].

Glutaraldehyde has high reactivity and high solubility in aqueous solution. It crosslinks PVA the same way as Glyoxal, improving, also, the mechanical strength of the gel. It favors the intermolecular reaction with PVA and is able to bind nonspecifically to proteins, being an attractive crosslinker for drug deliverey systems [17].

Due to the particular nature of the articular cartilage, the substitute biomaterial must provide not only the appropriate water content and biocompatibility but also adequate mechanical strength and tribological properties. So, it is necessary to evaluate such properties. Furthermore, it may be used as a drug delivery platform to help in the post-surgical recovery.

This work had two main objectives. The first, was to develop stable chemically crosslinked PVA-H, studying the effect of the concentration of different chemical crosslinking agents, glyoxal (G) and glutaraldehyde (GA), and the effect of PVA molecular weight (Low and Medium) on its tribomechanical properties. The second one was to evaluate the capacity of the material to be used as vehicle for local delivery of an anti-inflammatory, diclofenac.

2. Materials and methods

2.1. Materials

PVA with molecular weight of 145 000 g/mol and a degree of hydrolysis of 87%-89% and PVA with a molecular weight of 61 000 g/mol and a degree of hydrolysis of 87%-89% were purchased from Kuraray. 40% w/w aqueous solution of glyoxal was purchased from Alfa Aesar and 25% w/w aqueous solution of glutaraldehyde from Sigma-Aldrich were both used as crosslinkers. Diclofenac sodium salt was obtained from Sigma Aldrich was used in drug release.

2.2. Hydrogels preparation

7.75% w/V PVA solutions were prepared by dissolving the polymer in deionized and distilled water (DD water) in an oven at 90°C for 24 hours. For glyoxal containing hydrogels, after dissolution, the different amounts of glyoxal were added. The pH of the solution was adjusted to 4 with 400 μL of 1M HCL solution, to begin the crosslinking reaction. The solution was left at 80°C in a magnetic stirrer for 2 hours and after that neutralized to pH=7 with 1mL of 1M NaOH solution. To prepare the glutaraldehyde containing hydrogels, after dissolution of PVA, the following components were added: sulfuric acid, as a catalyst (1 ml, 1%), acetic acid as buffer (3 ml, 10%), methanol (2ml, 50%) as a quencher and glutaraldehyde. After addition of glutaraldehyde, mixing was performed at 105 rpm. All the solutions were then casted into petri dishes left at room temperature for 15h and then, placed in a oven at 37°C for the course of 4 days, and 2 days at 60°C. Table 1 shows the compositions of the produced PVA hydrogels (PVA-H).

Table 1: Scheme with the compositions of the PVA-H produced.

%w/v PVA	Mw (g/mol)	Crosslinker	%wt Crosslinker	Label
7.75 %	145 000	Glyoxal	10 %	PVA M + G10
			5 %	PVA M + G5
			1 %	PVA M + G1
			0.2 %	PVA M + G0.2
			0 %	PVA M
	61 000	Glyoxal	10 %	PVA L + G10
			5 %	PVA L + G5
			1%	PVA L + G1
			0.2%	PVA L + G0.2
		Glutaraldehyde	12%	PVA L + GA12
			6%	PVA L + GA6
			3%	PVA L + GA3
			1.5%	PVA L + GA1.5
			0.75%	PVA L + GA0.75
			0 %	PVA L

2.3. Hydrogels characterization

2.3.1 Swelling Behaviour

To measure the swelling capacity, the hydrated samples were cut with 8 mm of diameter and each disk was immersed in 1.5 mL of DD water and incubated at 36°C until their wet weight stabilized.Then, the samples were dried a 60°C and their dry weight was measured until the mass stabilized.

Knowing the dry and wet weights is possible to determine the equilibrium swelling ratio, (%ESR) and the Equilibrium Water Content (%EWC) with equations 1 and 2, respectively,:

$$\%\mathrm{ESR} = \frac{\mathrm{Wh\ -\ Wd}}{\mathrm{Wd}} \times 100 \quad (1)$$

$$\%\mathrm{EWC} = \frac{\mathrm{Wh\ -\ Wd}}{\mathrm{Wh}} \times 100 \quad (2)$$

2.3.2 Wettability

The Wettability of the produced hydrogels were determined by calculating the contact angle between the contour of a captive bubble (air) and the surface of sample immersed in DD water. The exeperimetns were performed in a captive bubble set-up with a JAI CV-A50 camera connected to a Data Translation DT3155) frame grabber and supported by a Wild M3Z optical microscope. 5 to 7 consistent bubbles were measured for each hydrogel.

2.3.3 SEM

The surface of samples was observed in Hitachi S-2400 scanning electron microscopy (SEM) with 15 KV voltage.

The samples were cut with 8 mm of diameter and placed in a -80ºC freezer for 2h and lyophilized for 48h to assure total removal of water. The polymeric surface of the hydrogels were coated with a 15 nm layer of Au/Pd by sputtering, using a Quorum Technologies sputter coater and evaporator.

2.3.4 HET-CAM

The irritation potential of the hydrogels were evaluated by HET-CAM test.

Each hydrogel disc was put on top of the chorioallantoic membrane of the eggs and observed for five minutes for signals of:

1. Bleeding (bleeding from vessels);
2. Vascular lysis (disintegration of blood vessels);
3. Coagulation (denaturation of intra- and extravascular proteins).

2.3.5 Drug Release

For the drug loading/release assays discs were cut with 11 mm of diameter, dried at 37º for 7 days and their dry weigth was measured.. Drug loading was performed by immersing the discs in 3 mL of drug solution, 2 mg/mL of diclofenac in Phosphate buffer solution (PBS)), for 3 days at 37ºC. For the release of diclofenac, the discs were immersed in 3 mL of drug solvent solution (PBS) and placed in a shaker (Incubating Mini Shaker from VWR) at 37ºC and 180 rpm.

The concentration of diclofenac released was quantified by measuring the solution's absorbance at 276nm using a spectrophotometer UV–VIS MultiscanGO from ThermoScientific® . All the release experiments were done, at least, in triplicate.

2.3.6 Mechanical Properties

Unconfined compressive and tensile tests were performed on a TA.XT Express Texture Analysis. For compressive tests, the samples, cut in squares with 5mm x 5mm, were immersed in DD water. The test was performed with a test speed of 0.1 mm/s and a force of 48 N. For tensile tests the samples were cut with a cutting stamp with specific dimensions. The test was performed at a constant strain rate (0.5mm/sec) with a force of 48 N. Prior to the assays the samples were always hydrated in DD water.A minimum of 3 repetitions per sample were done. Stress-strain curves were then plotted from which the elasticity modulus, fraction toughness were determined.

2.3.7 Tribological Properties

Friction sliding tests were performed on the hydrogels in a Tribometer TRM - Wazau, to obtain the coefficient of friction. A pin-on-dic set-up was used, with a pin of stainless stel (316L) with 6 mm of diameter and as lubricant, phosphate buffered saline (PBS) solution . The hydrogel samples were pre-equilibrated in PBS. The test was performed for six minutes at room temperature with an oscillation test mode, frequency of 2 Hz, sliding distance of 0.5 m, sliding velocity of 0.02 m/s and a friction radius of 23 mm and 19 mm.

3. Results & discussion

3.1. Preparation of PVA Hydrogels

Different crosslinkers and different amounts of the crosslinker were used to produce gels with different properties. However, the gels with an higher concentration of glyoxal, 5% wt and 10% wt, for both Mw of PVA, did not had the expected outcomes. The gels with with 5% wt (Figure 1 A), did not dissolved but were very fragile, gummy and disintegrated upon manipulation whereas the gels 10% wt when immersed in DD water, after drying, dissolved (Figure 1 B). Other authors have reported the reduction in crosslink density as a result of excessive addition of the crosslinker [17, 18]. Such degradation in crosslinking density was due to the reduction in the diffusivity of the reactant molecules. With increasing content of G, the solution became more viscous, which suppressed the diffusivity of the molecules and impeded the crosslinking reaction between PVA and glyoxal.

The polymeric mixture with fewer content of G resulted in homogeneous, flat transparent thin gels

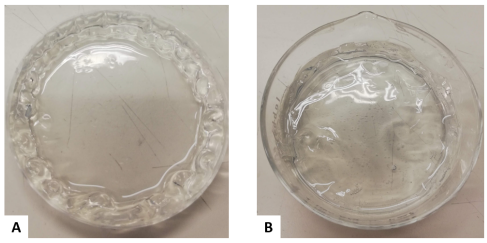


Figure 1: A: PVA hydrogel with 5% wt of glyoxal and B: PVA hydrogel with 10% wt of glyoxal

(Figure 2)

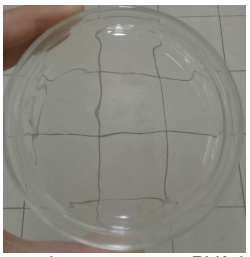


Figure 2: Transparent homogeneous PVA hydrogel with 0.2% wt of glyoxal

The hydrogels resulting from the addition of GA were quite different, as seen in Figures 3 and 4. The higher the concentration of GA, the more shrunk and rigid the final gel became (Figure 3 and 4), improving the water resistance of the films. This has been reported in literature [19].

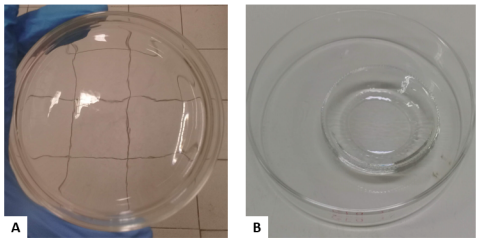


Figure 3: A: PVA hydrogel with 0.75% wt of GA and B: PVA hydrogel with 3% wt of GA.

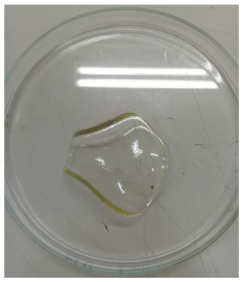


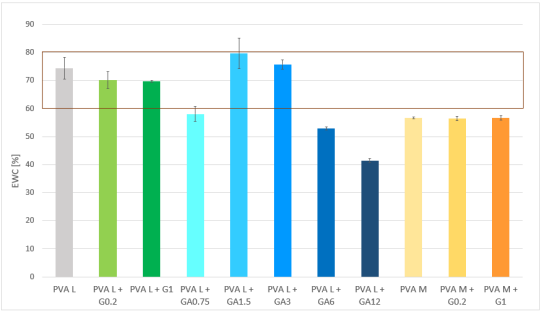
Figure 4: PVA hydrogel with 6% wt of GA

The hydrogels produced without adding crosslinkers were transparent and homogeneous, indicating its uniform network structure and distribution of microcrystallites. Nevertheless, the hydrogels produced with PVA of lower molecular weight were more thin and less rigid.

3.2. Characterization of PVA Hydrogels

3.2.1 Swelling Behaviour

The influences of PVA crosslinking and PVA's molecular weight on the water content and swelling degree of hydrogels immersed in DD water are shown in Figures 5 and 6, respectively.



The red box represents the range of WC values (60%-80%) for articular cartilage [20, 21].

Figure 5: Equilibrium Water content values of the different gels

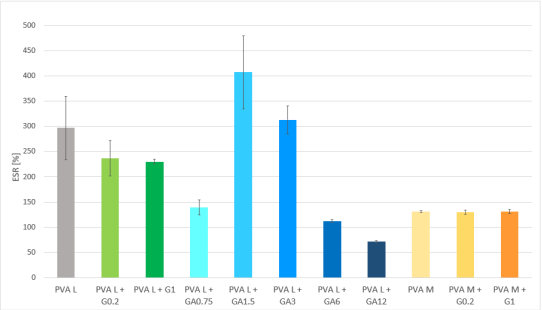


Figure 6: Values of the Swelling degree of the different gels.

In hydrogels with PVA of low molecular weight, the water uptake and swelling degree decreased with increasing weight percentage of glyoxal, because the free volume decreased owing to the more entangled polymeric network, improving water resistance. However, with higher PVA molecular weight the addition of G had little to no effect on the water content nor the swelling behaviour.

With the increase in PVA molecular weight, the heavier chains form a denser and tighter network of bond, resulting in a decrease in %EWC and %ESR.

In general, the addition of GA, decreases the %EWC and %ESR, with exception of the gels PVA L + GA1.5 and PVA L + GA3. It seems that GA in small amounts increased the affinity for water and thus increases swelling, whereas GA in higher amounts restrict the inter chain mobility, reducing flexibility and the available free space. Thus, increasing the membrane rigidity and water entrance resistance.

3.2.2 Wettability

To evaluate the surface wettability of the produced hydrogels, water contact angles were measured by the captive bubble method. In the results obtained, presented in Figure 7, contact angles are all generally low indicating that all hydrogels are highly hydrophilic. Moreover, the gels that have an higher degree of swelling have a lower contact angle, therefore, are more hydrophilic (compare Figure 5 and Figure 7).

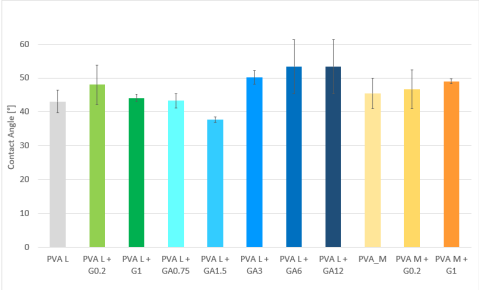


Figure 7: Contact angles obtained through Captive buble method.

The presence of G had little effect on the water contact angle, slightly increasing it, while the PVA's molecular weight did not affect the water contact angle. On average, the increase of GA content decreases the hydrogels wettability.

The values of the natural tissues found in literature [22, 23] are different than the ones obtained, probably because the method of measurement was different. In the sessile drop method the surface must be dry while in captive bubble the surface is equilibrated with the liquid.

The observed differences are typically explained by changes in the conformation of surfaces induced with drying. Besides, results are always prone to vary due to the surface irregularities.

Moreover, the contact angles of the PVA-H produced point out the hydrophilicity of the materials, and adequate values for the intended application.

3.2.3 Morphology

The surface's morphology of the produced PVA hydrogels analysed by SEM are presented in Figure 8.

The amount of G added had little influence on the surface's morphology. The presence of G, for both PVA molecular weight hydrogels led to smooth homogeneous surfaces without pores.

Once again, the influence of GA is more evident. These crosslinked PVA hydrogels revealed a lower porosity in all membranes with increasing GA content, as it has been reported in literature [24]. Increase in the PVA's molecular weight also decreases the presence of pores. The decrease on

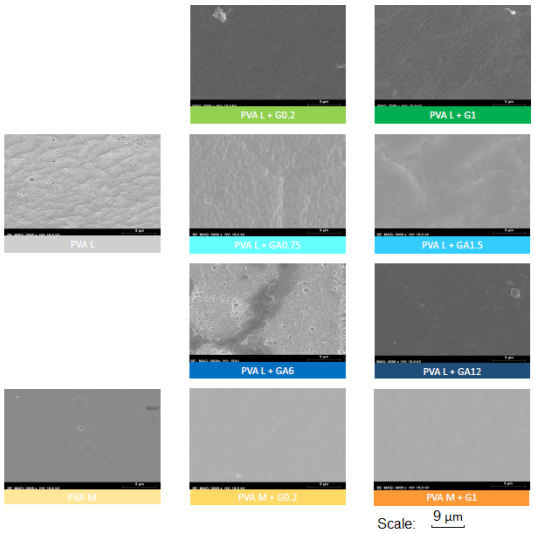


Figure 8: SEM images at 3000x of the different PVA hydrogels.

the porosity of the hydrogels justifies the decrease in the values of %EWC and %ESR.

The hydrogel PVA L + GA3 was not observed on the SEM, because prior to the SEM, compressive properties were analysed and that hydrogel did not resist the compressive forces applied, as it will be adressed in the next chapter

3.2.4 Mechanical Properties

From the compressive tests, were obtained the stress-strain curves shown in Figure 9 and, while Table 2 shows the mechanical properties values determined.

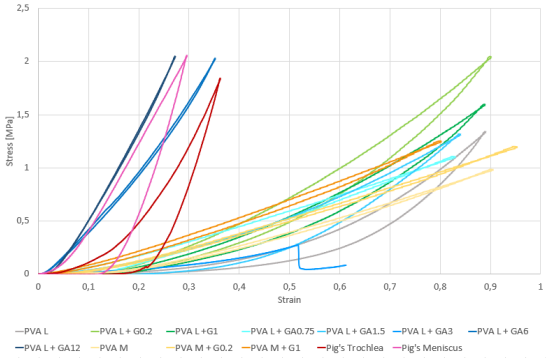


Figure 9: Compressive stress-strain curves of PVA-H.

There are no values for hydrogel PVA L + GA3, because it did not resist the compressive forces, due to its fragile matrix.

The addition of G leads to an increase of the elastic modulus, with the maximum value for the hydrogel PVA L + G0.2 (lighter green curve), and on the hydrogels with lower PVA's molecular weight, a slight increase on the energy dissipated.

The presence of GA also increased the elastic modulus. The highly concentrated GA hydrogels,

compared to other samples produced the far most dispersed results with the higher values of E (5,65 MPa for PVA M + GA6 and 8,82 MPa for PVA M + GA12). These results shows that the GA hydrogels are highly stiff and non-compliant materials, with negligible energy dissipated, thus, its response is entirely elastic, contrarily to pig's cartilage. Although, these hydrogels did not resist the tensile test, due to their fragile, vitreous like structure.

Increasing PVA's molecular weight increased the E, (hardened the hydrogels) and also reduced the energy dissipated, resulting in a response mainly elastic. The presence of G in these hydrogels contributed to the increase of E, with maximum values for PVA M + G1. However, the values of dissipated energy and the energy of recovery did not suffer significant changes.

Table 2: Summary of the compressive properties of the PVA-H produced.

Material	Compressive elastic modulus strain [0.3-0.4] (MPa)	Compressive elastic modulus stress [0.6-0.9] (MPa)	Energy of recovery (MJ/m^3)	Energy dissipated (MJ/m^3)
PVA L	0.76 ± 0.06	2.66 ± 0.27	0.31 ± 0.04	0.08 ± 0.006
PVA L + G0.2	2.07 ± 0.11	2.63 ± 0.14	0.49 ± 0.05	0.15 ± 0.03
PVA L + G1	1.55 ± 0.03	2.19 ± 0.23	0.47 ± 0.03	0.09 ± 0.02
PVA L + GA0.75	1.45 ± 0.12	1.54 ± 0.1	0.41 ± 0.06	0.05 ± 0.006
PVA L + GA1.5	1.52 ± 0.07	1.94 ± 0.21	0.35 ± 0.03	0.11 ± 0.03
PVA L + GA6	5.64 ± 0.28	5.19 ± 0.36	0.27 ± 0.02	0.007 ± 0.002
PVA L + GA12	8.82 ± 1.2	8.67 ± 1.03	0.22 ± 0.03	0.003 ± 0.001
PVA M	1.64 ± 0.32	1.70 ± 0.21	0.34 ± 0.05	0.02 ± 0.005
PVA M + G0.2	1.70 ± 0.45	1.73 ± 0.63	0.40 ± 0.05	0.02 ± 0.002
PVA M + G1	1.80 ± 0.08	1.87 ± 0.05	0.40 ± 0.01	0.03 ± 0.003

From the results obtained for the %EWC and %ESR, these findings were expected. In general, those who presented lower water content have better mechanical performance.

As it evidenced in Figure 9, most of the hydrogels curves are far more to the right than those of pig's cartilage, meaning that they deform more easily under load.

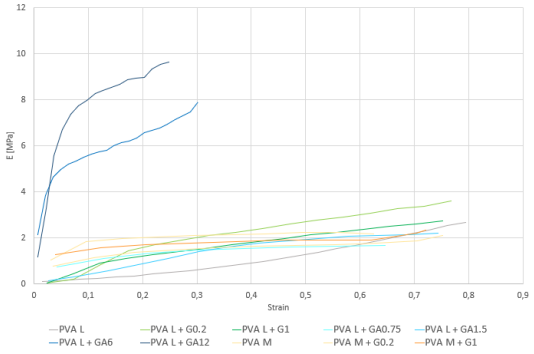


Figure 10: E vs. strain on PVA-H under compression.

The variation of E with strain is displayed in Figure 10. An important property of viscoelastic materials is that their mechanical properties depend on the rate at which they are deformed. The stiffness of the material increases with the loading rate, as is observed in Figure 10, where E, for all samples, increases.

From tensile tests, stress-strain curves were obtained, as seen in Figure 11 and the data calculated is summarized in Table 3.

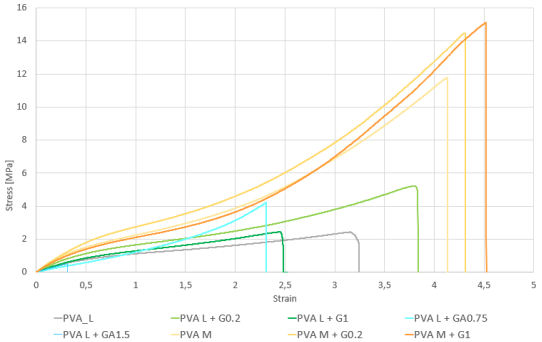


Figure 11: Tensile stress-strain curves of PVA-H.

Table 3: Summary of the tensile properties of the PVA-H produced.

Material	Elastic Modulus strain [0.3-0.4] (MPa)	Elastic Modulus stress [0.7-1] (MPa)	Ultimate tensile strength (MPa)	Tensile toughness (MJ/m^3)
PVA L	1.15 ±0.09	0.78 ± 0.15	2.76 ± 0.88	5.21 ± 1.9
PVA L + G0.2	1.75 ± 0.14	1.80 ± 0.30	4.58 ± 0.94	9.82 ± 0.25
PVA L + G1	1.26 ± 0.13	0.90 ± 0.23	2.85 ± 0.83	5.54 ± 3.81
PVA L + GA0.75	1.69 ± 0.64	1.88 ± 0.89	3.94 ± 2.04	4.85 ± 2.77
PVA L + GA1.5	1.26 ± 0.59	1.59 ± 0.39	0.42 ± 0.2	0.09 ± 0.05
PVA M	2.35 ± 0.12	3.09 ± 0.20	13.81 ± 2.98	23.9 ± 6.17
PVA M + G0.2	3.07 ± 0.42	3.21 ± 0.67	14.58 ± 2.73	26.71 ± 7.93
PVA M + G1	3.28 ± 0.06	4.52 ± 1.17	12.68 ± 2.26	30.04 ± 5.29

The addition of crosslinkers (G and GA) increases significantly the elastic modulus, due to the increased network density, toughness and the UTS, with exception of PVA L + GA1.5 that had values even lower than the blank hydrogel. Increasing PVA molecular weight highly increases the E, UTS and toughness, because the more dense and closed polymeric matrix, increasing the rigidity of the hydrogels.

Natural cartilage Young's modulus values show that it is stiffer when subjected to tensile forces and softer under compressive loads [5]. This allows the release of synovial fluid, upon compression, enabling an efficient lubrication on a cushion effect.

This behaviour was only observed in hydrogels with higher PVA molecular weight, although the E values under tensile loads were still low when compared to natural cartilage, shown in Figure 12.

The calculated compressive and tensile E were slightly different in the tested hydrogels, which has

already been reported in literature [25]. The E is dynamic, and might be affected by the type of load (compressive or tensile), the strain magnitude and rate, the temperature and the fiber orientation, indicating a non linear viscoelastic behaviour. Anisotropy might be another reason why PVA behave differently when upon tensile and compressive stresses therefore, the results here presented seem to support the idea that both the PVA and the cartilage are anisotropic materials.

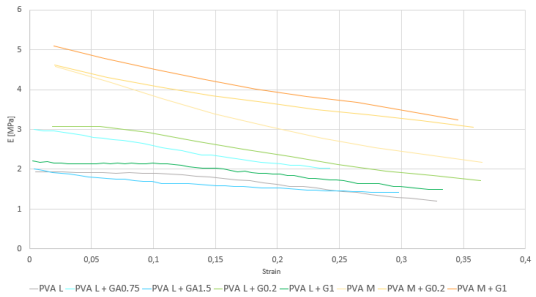


Figure 12: E vs. strain on PVA-H under tension.

The ranking of the E values, under tension, for each sample is in agreement with the values calculated and presented in Table 3. while stretching, the free space between molecules increases, decreasing the resistance of the hydrogel to the tensile forces, thus, decreasing the E.

3.3. Tribological Properties

Loads of 10N and 20 N were applied to the hydrogels, originating contact pressures of (1 MPa and 2 MPa). Natural cartilage is subjected to contact stresses of approximately 1–5 MPa [26, 27, 28] under normal conditions.

The influence of the studied variables on the coefficient of friction (μ) of PVA-H, are shown in Figure 13.

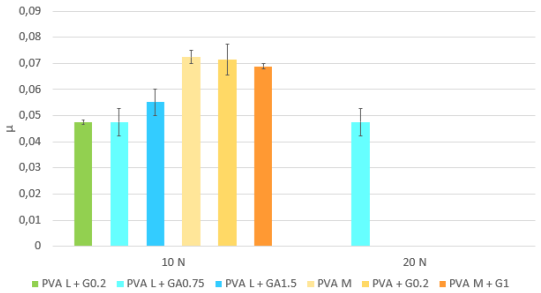


Figure 13: Variation of the coefficient of friction with applied load on the PVA-H hydrated in PBS.

When carrying out tribological tests, hydrogels must undergo deformation and as a result, part of the water inside the polymeric matrix will be released into the contact zone between the surfaces acting as a lubricant. Thereby, increased thickness of the existing liquid film between the two surfaces in contact will occur, contributing to a low μ.

There are no values of the coefficient of friction for PVA L and PVA L + G1 because the load of 10 N was enough to make a hole in the sample. Most of the hydrogels only hang on a load of 10 N. The maximum load applied to the hydrogels was 20 N, but only PVA L + GA0.75 was able to resist without break.

For the hydrogels with lower PVA molecular weight, the addition of G and GA improved its resistance to frictional forces. It shall be stressed, that there was no significant difference on the values of μ on the hydrogels that resisted the loads applied. Moreover, the addition of G was only benefic in its lower percentage, which is in line with the results for mechanical resistance and water content. PVA L + GA0.75 showed the highest resistance to frictional loads. However, at 20 N, the coefficient of friction remain the same. Only an increase in the standard deviation that can be due to the heterogeneity of the material itself. Increased GA content increased the μ, without increasing its resistance to higher frictional loads, as shown in Table 4.

Increasing PVA molecular weight, increases the μ. The addition of G on these hydrogels slightly decreased the μ, but also did not improved the hydrogel's resistance to higher loads.

Table 4: Summary of the wear results from the tribological tests.

Material \ Load	Wear at **10N**	Wear at **20N**
PVA L + G0.2	Visible wear	-
PVA L + GA0.75	No wear	No wear
PVA L + GA1.5	Minimum Wear	-
PVA M	Visible Wear	-
PVA M + G0.2	No wear	-
PVA M + G1	No wear	-

It is worthwhile mention that the thin thickness of the hydrogels can be the reason for the low wear resistance observed. Nevertheless, the addition of crosslinkers improved the gel's resistance to wear as demonstrated in Table 4.

Although the experimental conditions of the tribological tests, reported in the literature for natural cartilage and PVA hydrogels vary from case to case, the coefficients of friction obtained in this work are within the range observed for natural cartilage, 0.01-0.25 [2, 29, 30, 31, 32] , and other PVA hydrogels, 0.04-0.07 [29]. However, the formation of wear trails in some samples, at low loads, demonstrates poor wear resistance of those materials.

3.4. Irritation Potential

The HET-Cam test indicated that all samples are not irritating. Since the chorioallantoic membrane does not have signs of haemorrhage, coagulations and/or vessel lysis after 5 minutes in contact with

the sample. Therefore, the hydrogels should be suitable to be used in contact with human tissue.

3.5. Drug Release

Drug release experiments were carried out on the selected hydrogels to evaluate if they could be an effective platform for drug delivery and to compare the effect of the crosslinkers on the drug release behaviour, in terms of release kinetics and amount of drug released. The release profiles from PVA L + G0.2 and PVA L + GA0.75 are presented in Figure 14 and for PVA M + G1 in Figure 15.

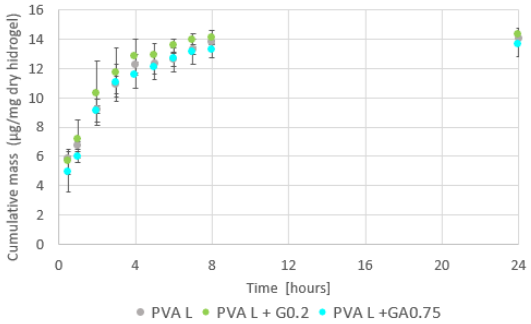


Figure 14: Cumulative release profiles of DFN from PVA L + G0.2, PVA L + GA0.75 and the respective non-crosslinked sample. The error bars correspond to ± SD mean.

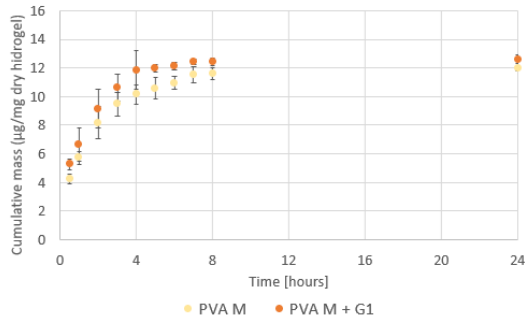


Figure 15: Cumulative release profiles of DFN from PVA M + G1 and the respective non-crosslinked sample. The error bars correspond to ± SD mean.

In all samples, there was an initial burst in the released drug and almost all drug was released in the first 24 h.

Addition of crosslinkers did not affect significantly the drug release profile of the hydrogels. The amount of drug release decreased with increasing molecular weight of PVA Figure 15, which may have been due to an increased diffusional path length for the solute, caused by the more tight and closed polymeric matrix, but also led to a rapid release. Polymeric matrices with lower %EWC values loaded less drug.

4. Conclusions

In general, the hydrogels were prepared successfully. However, hydrogels with high amounts of glyoxal (5% wt and 10 % wt) did not form stable materials.

Similarly to natural cartilage, all the produced hydrogels are hydrophilic, most of them presented an high degree of swelling, being the most visible exception PVA L + GA6 and PVA L + GA12, that presented lower values, and low coefficient of friction due to its biphasic nature allowing the release of water from the polymeric matrix increasing the lubricant film between the surfaces.

The increase in PVA molecular weight improved the mechanical properties. In general, the addition of crosslinkers, also improved the mechanical properties, due to the reinforced entangled network. For lower PVA molecular weight, lower amounts of G and GA were more beneficial, while for higher PVA molecular weight, higher amount of G produced the best results. Higher amounts of GA produced highly stiff and non-compliant hydrogels, that were fragile under tension. However, the mechanical properties of the hydrogels remain inferior to those of natural cartilage, with the hydrogels deforming easily under load.

Although the low coefficient of friction ($<$0.08), the hydrogels did not resist loads above 10 N. The HET-Cam test validated all samples as non irritating, allowing their contact with human tissue.

The addition of crosslinkers (glyoxal and glutaraldehyde) did not significantly affected the drug release profile of the hydrogels. The increase in PVA's molecular weight restrain the drug incorporation and consequently decreased the quantity of drug released.

5. Future work

Investigation on methods to produce more stable GA crosslinked hydrogels should be carried out, since these had very interesting but varied outcomes. One strategy would be combining it with higher molecular weight PVA. Slightly increase on PVA content should also be carried out in order to improve the gel's mechanical and tribological properties and to produce thicker gels.

Furthermore, it would be advantageous to study the effect of relevant lubricants in the tribological tests, like hyaluronic acid, since it is present in synovial fluid. Also to perform a more complete drug release assay, comparing larger number of samples and adding other compounds, like vitamin E, that is known to delay the release of the drug.

Finally, the study of the effects of sterilization methods on the tribomechanical properties should be addressed, since in order to introduce the material in the human body it is a mandatory step, and it may modify the materials properties. So, it would be interesting to study what are the effects of the available sterilization methos over the hydro-

gels properties.

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