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Physicochemical characterization of barrier membranes for bone regeneration

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ABSTRACT

Barrier membranes are essential biomaterials for guided bone regeneration. Due to different origin and structure of barrier membranes, singular mechanical properties and clinical behaviors can be expected. It is important to understand the physic and chemical properties of barrier membranes to select the needed biomaterial for each clinical situation. To date, no study has evaluated and compared the physicochemical properties of various families of barrier membranes. The aim of this study is to evaluate the physicochemical properties of various barrier membranes. Fifteen membranes of different origin were tested in this study. Membranes were divided into biological or synthetic origin and grouped in natural allogenic collagen, natural xenogenic collagen, cross-linked collagen and synthetic membranes. Physicochemical properties were evaluated in terms of tension, stiffness, absorption ability, pH and wettability. For the tension tests, all membranes showed similar low tension and low stiffness, especially after a 4-min hydration, except for bone laminas that showed a greater stiffness particularly in a dry status. Regarding wettability and hydration of the barrier membranes, porcine origin membranes had greater hydration; wettability was also superior in porcine derived barrier membranes and showed a faster absorption of the drop on the rough surfaces. All membranes had a stable pH, having the synthetic membranes the most stable pH when compared to physiologic. The wide variety of barrier membranes opens a debate in which the practitioner should select the adequate barrier membrane for each clinical situation. Different materials show singular potentials depending on their tissue origin making them suitable for specific clinical indications. More studies regarding adsorption, integration and degradation of barrier membranes are needed to understand their behavior.

1. Introduction

Guided Bone Regeneration (GBR) and Guided Tissue Regeneration (GTR) techniques mandate the use of a barrier membrane (Dahlin et al., 1988; Pontoriero et al., 1992). The main function of barrier membranes is to separately guide the regeneration of soft and hard tissues, avoiding the ingrowth of epithelium and connective tissue in the bone compartment (Bornstein et al., 2007; Rakhmatia et al., 2013; Zitzmann et al., 1997). There is currently a wide range of membranes, which are generally classified according to their origin, mainly allogenic, xenogenic or alloplastic origin (Caballe-Serrano et al., 2018) or according to their degradation, existing resorbable and non-resorbable membranes (Bunyaratavej and Wang, 2001; Rakhmatia et al., 2013). Furthermore, classifications based on the material of origin also exist (Hutmacher et al., 1996). Despite of its classification, it is important to understand the physicochemical and biological properties related to the barrier membranes to comprehend their behavior in clinical scenario.

Regarding the mechanical properties of barrier membranes, a consensus has been recently established showing that a balance must be set between the mechanical stability and the stiffness to ensure proper regeneration (Elgali et al., 2017). While the minimum mechanical load of the membrane supports is directly related with its resistance to fracture and deformation, a low elastic behavior of the membrane may permanently deform the structure and hence reduce its proper functionality. Special applications might require membranes with a high Young Modulus to maintain their shape (Wachtel et al., 2013) while

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other might need more elastic and flexible membranes to adapt better to the defect and stabilize the bone filler (Urban et al., 2013).

Besides the mechanical stability of the membranes, it is important to understand how membranes will behave in contact with biological fluids. The absorption ability of the barrier membranes will greatly vary depending on their origin and composition (Sanders and Kingsnorth, 2012). Natural origin membranes, having a more hydrophilic composition, generally absorb faster and greater amounts of liquid compared to synthetic membranes. In a similar way, as the membrane is wet, a series of electrostatic interactions take place, varying the electrostatic charge of the fluid as well as the pH, at short times, as a consequence of the inherent physical properties of the membrane (O'Brien, 2011). These initial electrostatic interactions are stabilized after initial moments due to the buffering effect of physiological fluids. Nevertheless, this initial phase may play a pivotal role in the subsequent tissue regeneration and cellular interaction. For instance, it is known that low pH values may influence the appearance of macrophages, which are directly bound to inflammatory processes (Gerry and Leake, 2014). At longer time points, it is also well established that fluctuations in the pH can affect the degradation and the interaction of the biomaterials with the surrounding cells (Ruan et al., 2017; Ulery et al., 2011). Overall, the possible electrostatic interactions are based on the surface properties and chemical composition which in the end orchestrate the several biological processes that take place in clinical scenarios (Miron et al., 2017). In this sense, the surface energy and hydrophilicity of materials, which is measured with a contact angle technique, offers a comprehensive system to analyze these properties, showing that lower contact angle surfaces are more prone to enhanced biocompatibility and tissue integration, as opposed to higher contact angles that reduce cell adhesion and hence tissue regeneration (Dowling et al., 2011; Schieber et al., 2017; Schwarz et al., 2009).

In the present study, the objective is to compare different commercially available barriers membranes and analyze their mechanical and physicochemical properties in order to have a database of the general functional properties of the membranes. The intention is to broaden the knowledge in the regeneration field and clarify their clinical use.

2. Material and methods

2.1. Membrane specifications

The membranes used are summarized in Table 1. Specifications of all barrier membranes studied were obtained from the manufacturer's technical data.

2.2. Tensile test

Tensile test was performed using a tensile tester (Instron, ITW Company) equipped with a load cell with a maximum range of a 100N. Barrier membranes were cut into sections of 5 mm by 15 mm and placed on a custom-made mounting plate. The central 5 mm of the sections were protected by a bar and the loose ends of the section were embedded in a self-curing epoxy resin. The resin embedded portion of the membranes were clutched by the handles of the tensile tester. The square central portion of the sections had 25 mm^2 and was free of resin. Tests were performed in double triplicates using membranes from different lots. Tensile force was expressed as tension (MPa). Data were reported comparing each membrane in a dry state, 2 min and 4 min hydrated with distilled water. Stiffness of membranes on a wet state (4 min) was evaluated calculating the Young Modulus of the linear elastic portion of the deformation graph, thickness of membranes was estimated to 1 mm.

Table 1
Specifications of the barrier membranes used according to the information provided by the manufacturer online or on the product datasheet: origin, family material, subfamily material, composition, product name, manufacturer and thickness.

Origin	Family material	Subfamily material	Composition	Product name	Manufacturer	Thickness
Biologic Origin	Allogenic Collagen	Natural allogenic collagen	Acellular dermal matrix	Alloderm thin GBR	LifeCell	0,9–1,6 mm thick (GTR)/0,5–0,9 mm thick (GBR)
	Xenogenic collagen	Natural collagen	Porcine peritoneum collagen I&III	BioGide	Geistlich Pharma	Non information available
			Porcine dermis	Collprotect	Botiss Biomaterials GmbH	~ 0,4 mm thick
			Porcine pericardium	Jason	Botiss Biomaterials GmbH	~ 0,15 mm thick
			Porcine tendon collagen I	Collagen P	Genoss Co	0,3 mm thick
			Porcine cortical bone	Lamina (Porcine)	OsteoBiol® (Tecnoss®)	0,5 mm thick, 0,8-1 mm thick, 2–4 mm thick (fine, medium curved and standard)
			Equine pericardium	Evolution	OsteoBiol® (Tecnoss®)	Fine 0,3 mm thick, standard 0,5 mm thick
			Equine cortical bone	Lamina (Equine)	Osteobiol (Tecnoss Dental)	0,5 mm thick, 0,8-1 mm thick, 2–4 mm thick (fine, medium curved and standard)
			Bovine atelo-collagen type I	ImploSorb	Bioimplon GmbH	0,3 mm thick
			Bovine tendon collagen I + Hyaluronic acid	ImploFlex	Bioimplon GmbH	0,3 mm thick
Synthetic origin	Resorbable	Cross-linked by formaldehyde	Bovine collagen I	Cytoplast RTM	Collagen Matrix US	Non information available
		Cross-linked by riboses	Porcine collagen	Ossix Plus	Datum Dental	Non information available
		Polycaprolactone	Polycaprolactone (PCL)	Osteoguide	Genoss Co	0,12 mm thick
		polylactic-Glycolic Acid (PLGA)	Polylacti-Glycolic Acid (PLGA)	Tisseos	Biomedical Tissues	0,4 mm thick
	Non-resorbable	PTFE-dense	PTFE	Cytoplast TXT	Osteogenics Biomedical	Non information available

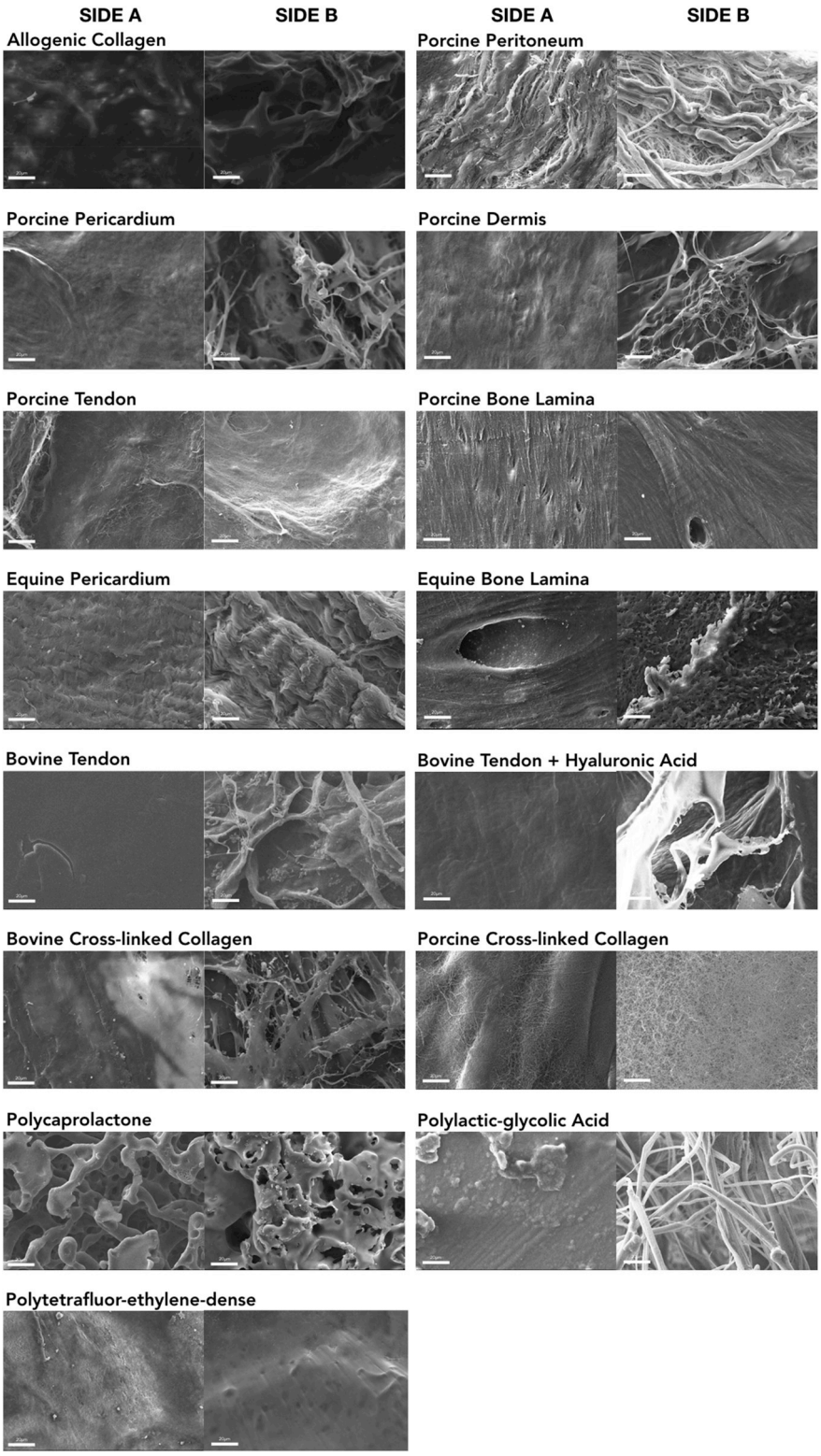

Fig. 1. A. SEM Images of the barrier membranes tested. Images show Side A (smooth) and Side B (rough) surface respectively. Scale bars represent 20 μm. B. Macroscopic images of the barrier membranes after flexural strength test. Samples are analyzed after 4 min hydration. Scale bars represent 1 mm.

2.3. Image acquisition

For scanning electron microscopy (SEM) images, samples were metal coated withan alloy of 80% gold - 20% palladium with a thickness of 10–20 nm and observed in high vacuum on a Zeiss 940 DSM scanning electron microscope. Images were taken for both sides in order to analyzed the rough and smooth surface of the membranes. Macroscopic images of barrier membranes were taken of hydrated membranes during 4 min after tensile test. For this purpose, a stereo loupe microscope (Zeiss Stereo Discovery V8) and a ZEN 2 Software (2011) were used.

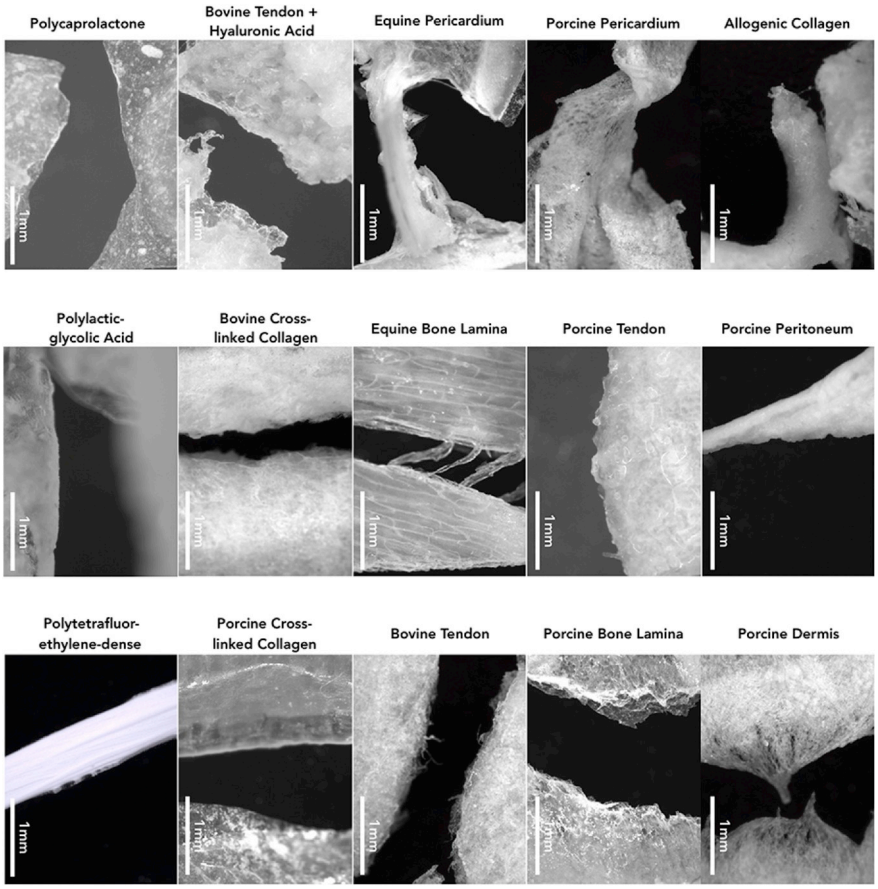

Fig. 1. (*continued*)

2.4. Absorption ability

The absorption ability of membranes was performed hydrating standardized samples of membranes during 6 min with a Phosphate Buffer Solution (PBS) with a pH of 7,4. All membranes were soaked in 1 ml of PBS. Data was recorded at 2 min, 4 min and at 6 min of hydration. No further time points were selected because the plateau of all membranes was reached after 4 min. Each sample was weighed in a dry state and at every time point. Increase of weight was normalized to the initial dry sample weight. The absorption ability is expressed normalized in fold-increase. Tests were performed in double triplicates using membranes from different lots. In order to understand fluctuations in the pH of the PBS immersed in contact with the different membranes, after the complete soaking, the remaining solution was measured with pH-meter (Mettler Toledo™ FiveEasy™) to record pH value.

2.5. Wettability

To analyze surface energy and wettability, static contact angle of the rough and the smooth surface of barrier membranes was measured by the sessile drop method and analyzed using Contact Angle Meter (Contact AngleSystem OCA 15 plus, Dataphysics, Germany). Drops of 3 µL were generated with a micrometric syringe on the surface of the membranes using mili-Q water. Contact angle was measured at 4 s and 30 s after applying the Laplace-Young fitting of the drop profile with SCA 20 software (Dataphysycs). Test was performed in triplicates for each group using membranes from different lots.

2.6. Physicochemical index and properties summary

A radial graph of 5 ends was created summarizing the data of all experiments. Data of tension in wet conditions, stiffness in wet conditions, wettability, hydration (after 6 min) and the pH value after complete hydration. To represent the data in the graph, each membrane values were normalized to the parameters that we believe need to be either maximized or minimized. Tension for each membrane was normalized to the highest obtained (Equine Bone Lamina); wettability values of the rough side were normalized to lowest contact angle (Porcine Peritoneum and Porcine Cross-Linked Collagen); hydration values were normalized to the membranes with the highest absorption (Porcine Cross-Linked Collagen). The pH values were represented as follows: physiologic pH of 7,4 was set as 100%, representing any deviation with higher or lower values appear with a score below 100%. Stiffness data (tension data) was represented in two ways: data normalized to the lowest Young Modulus (Polycaprolactone) and highest Young Modulus (Porcine Bone Lamina). This difference was done since in some real clinical scenarios lower stiffness is desired whereas in other scenario higher stiffness is desired. The normalized values were then plotted in the pentagon radial graph, presenting the two different superimposed graphs for the two different stiffness values.

To calculate the physicochemical index, total area of the pentagon and the area of the different graphs was determined using Image J Software (NIH, United States of America). Area of the different graphs was normalized to the total area of the pentagon and expressed in a scale of 1–10, being 10 the total area of the pentagon.

2.7. Statistical analysis

A descriptive analysis has been performed to study the mean and standard deviation. To perform a statistical analysis, Mann-Whitney test has been utilized to compare the distribution of the values between two independent samples. When more than two samples had to be compared simultaneously, Kruskal-Wallis test was performed. Post-hoc comparisons between different levels were corrected according to

Bonferroni. The level of significance employed was set at 5% (p = 0,05). To perform the statistical analysis the following groups were confronted: each porcine vs each porcine, each bovine vs each bovine, each equine vs each equine, each alloplastic vs each alloplastic, allogenic dermis vs porcine dermis, porcine pericardium vs equine pericardium, porcine bone lamina vs equine bone lamina and porcine cross-linked vs bovine cross-linked. Exact p-values of each comparison are collected in Supplement document 1.

3. Results

A summary of the results is shown in each of the tables and figures. The tables have been presented in such a way that highest and lowest values are easily identified by the colours scale. Statistical comparisons have been arranged according to their origin (animal or tissue) as we consider that comparing all different membranes among each other has little relevance. Furthermore, relevant significant differences have been incorporated within the text, but not within the tables in order to clearly present the results. All p-values can be found in Supplement document 1.

3.1. Microstructure of membranes

Microstructure varied among membranes depending on their tissue of origin (Fig. 1(a)). Porcine natural collagen membranes, exhibited similarity on their rough surface except for the Porcine Tendon and the Porcine Bone Lamina, which presented more compact collagen fibres, presenting a similar morphology on both sides. Collagen fibres diameter were thinner in the Porcine pericardium membranes than in the other porcine samples.

Collagen fibres from Equine Pericardium membranes were more dense than Porcine Pericardium membranes but not as compact compared to Equine Bone Lamina. Bovine Tendon membrane presented thicker but less compact fibres than Porcine Tendon membrane. When comparing the smooth surface of Bovine membranes, surface was smoother and more homogenous to any other membrane type. Bovine Tendon membrane implemented with hyaluronic acid presented aggregated collagen fibres on the rough surface.

Cross-linked collagen membranes exhibited different microstructure compared to all other membranes where Bovine Cross-linked Collagen with formaldehydes showed thicker fibres than non-cross-linked bovine membranes or Porcine Cross-linked Collagen membranes. Contrary to this finding, Porcine Cross-linked Collagen membranes presented thin collagen fibres with higher density.

Macrostructure of alloplastic membranes presented different morphology than collagen membranes. Polycaprolactone (PCL) membrane exhibited porous structure with delimited fibres with a spongy shape. Polylactic-glycolic Acid (PLGA) membranes presented homogeneous surface on the smooth surface and numerous dense fibres on the rough surface. Polytetrafluorethylene –dense (PTFE) membranes presented comparable surface of the smooth and the rough surface with a homogeneous appearance.

Table 2 (a)
Mechanical properties expressed in terms of tension (MPa) of the barrier membranes. The table shows the mean and standard deviation in a dry status, two and 4 min hydration respectively. Collagen membranes presented the higher tension values. Dark blue colours were given to the higher tension values whereas light blue colours were given to the lower. Four blue colours are used in total.

Membrane	Tension (MPa)		
	Dry Mean ± SD	2' Mean ± SD	4' Mean ± SD
Allogenic Collagen	1,3 ± 0,4	0,6 ± 0,1	0,8 ± 0,1
Porcine Peritoneum	0,4 ± 0,1	0,1 ± 0,0	0,1 ± 0,0
Porcine Dermis	0,7 ± 0,1	0,3 ± 0,2	0,3 ± 0,2
Porcine Pericardium	0,2 ± 0,1	0,2 ± 0,1	0,1 ± 0,1
Porcine Tendon	1,8 ± 0,3	0,4 ± 0,1	0,2 ± 0,1
Porcine Bone Lamina	2,1 ± 0,7	0,4 ± 0,1	0,8 ± 0,3
Equine Pericardium	1,2 ± 0,3	0,5 ± 0,1	0,4 ± 0,1
Equine Bone Lamina	1,7 ± 0,3	0,7 ± 0,2	0,9 ± 0,3
Bovine Tendon	0,8 ± 0,1	0,0 ± 0,0	0,0 ± 0,0
Bovine Tendon + Hyaluronic Acid	0,6 ± 0,1	0,0 ± 0,0	0,0 ± 0,0
Bovine Cross-linked Collagen	0,6 ± 0,3	0,2 ± 0,1	0,1 ± 0,0
Porcine Cross-linked Collagen	0,5 ± 0,4	0,1 ± 0,1	0,2 ± 0,0
Polycaprolactone	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Polylactic-Glycolic Acid	0,1 ± 0,1	0,0 ± 0,0	0,0 ± 0,0
Polytetrafluorethylene-dense	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,1

Table 2(b)
Mechanical properties expressed in terms of Young Modulus of the barrier membranes. Note a generalized decrease of the young modulus after hydration, specially in collagen membranes. Dark blue colours were given to the higher young modulus values whereas light blue colours were given to the lower. Note that a low young modulus denotes more flexibility than membranes with a high young modulus. Four blue colours are used in total.

Membrane	Young Modulus		
	Dry Mean ± SD	2' Mean ± SD	4' Mean ± SD
Allogenic Collagen	0,4 ± 0,1	0,3 ± 0,1	0,3 ± 0,0
Porcine Peritoneum	0,1 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Porcine Dermis	0,6 ± 0,3	0,2 ± 0,0	0,2 ± 0,1
Porcine Pericardium	0,4 ± 0,0	0,1 ± 0,0	0,2 ± 0,0
Porcine Tendon	0,1 ± 0,0	0,0 ± 0,0	0,1 ± 0,0
Porcine Bone Lamina	4,8 ± 0,4	0,5 ± 0,1	0,9 ± 0,1
Equine Pericardium	2,2 ± 0,1	0,6 ± 0,1	0,5 ± 0,1
Equine Bone Lamina	3,8 ± 0,1	0,7 ± 0,2	0,2 ± 0,0
Bovine Tendon	0,7 ± 0,1	0,0 ± 0,0	0,0 ± 0,0
Bovine Tendon + Hyaluronic Acid	1,8 ± 0,1	0,0 ± 0,0	0,0 ± 0,0
Bovine Cross-linked Collagen	0,4 ± 0,0	0,6 ± 0,1	0,4 ± 0,1
Porcine Cross-linked Collagen	1,3 ± 0,1	0,3 ± 0,1	0,4 ± 0,1
Polycaprolactone	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Polylactic-Glycolic Acid	0,2 ± 0,1	0,0 ± 0,0	0,1 ± 0,1
Polytetrafluorethylene-dense	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0

3.2. Tensile test

Depending on the tissue origin, membranes presented different tension patterns (Table 2(a)). A significant difference existed between dried membranes and all other groups with a p-value less than 0,05. Dried Porcine Bone Lamina, exhibited the highest tension values with 2,1 MPa before rupture followed by dry Porcine Tendon (1,8 MPa) and dry Equine Bone Lamina (1,7 MPa). When membranes were hydrated 2 and 4 min, tension at fracture decreased except for the Allogenic Collagen membrane that withstood high values of tension despite being wet. Alloplastic membranes showed lower tension to fracture with values compared to collagen membranes up to 0,1 MPa before rupture. Exact p-values of each comparison are collected in Supplement document 1.

Macroscopical images of the rupture pattern of membranes after tensile test showed that configuration of collagen fibres affects the rupture pattern (Fig. 1(b)). Membranes with dermal origin (Allogenic Collagen and Porcine Dermis), suffered less elongation before rupture compared to pericardium membranes. Membranes from tendons showed an abrupt rupture without the elongation of the fibres, same as laminas, cross-linked membranes, PCL and PLGA membranes. PTFE membrane presented the most elongation followed by the Porcine Peritoneum membrane.

Young Modulus analysis revealed that alloplastic membranes are less stiff than all other Collagen membranes with a statistical significance. When Collagen membranes were hydrated after two and 4 min, the stiffness decreased. All porcine membranes showed statistical significant differences between them at all time points (Table 2 (b)).

Table 2(c)
Absorption ability of the barrier membranes. Mean of the absorption ability and standard deviation. All membranes reached the plateau values within 6 min. Dark blue colours were given to the higher absorption values whereas light blue colours were given to the lower. Four blue colours are used in total.

Membrane	Absorption (x-fold increase)		
	2' Mean ± SD	4' Mean ± SD	6' Mean ± SD
Allogenic Collagen	2,6 ± 0,4	2,8 ± 0,4	2,7 ± 0,3
Porcine Peritoneum	5 ± 0,5	5,8 ± 0,1	6,0 ± 0,3
Porcine Dermis	5,4 ± 0,6	5,4 ± 0,7	6,3 ± 0,7
Porcine Pericardium	7,1 ± 2,5	7,9 ± 2,1	8,2 ± 2,2
Porcine Tendon	2,4 ± 0,1	1,6 ± 1,0	2,6 ± 0,0
Porcine Bone Lamina	1,9 ± 0,1	2,2 ± 0,1	2,3 ± 0,1
Equine Pericardium	4,3 ± 0,9	4,2 ± 0,9	4,6 ± 1,0
Equine Bone Lamina	1,8 ± 0,1	2,1 ± 0,1	2,2 ± 0,2
Bovine Tendon	3,1 ± 0,7	3,7 ± 0,2	4,1 ± 0,2
Bovine Tendon +Hyaluronic Acid	3,5 ± 0,4	4,5 ± 0,3	4,9 ± 0,1
Bovine Cross-linked Collagen	4,3 ± 0,4	4,4 ± 0,3	4,4 ± 0,4
Porcine Cross-linked Collagen	8,5 ± 1,3	9,2 ± 1,4	9,5 ± 1,5
Polycaprolactone	1,2 ± 0,1	1,1 ± 0,1	1,1 ± 0,1
Polylactic-Glycolic Acid	1,9 ± 0,3	2,6 ± 1,1	2,9 ± 1,1
Polytetrafluorethylene-dense	1,1 ± 0,1	1,0 ± 0,0	1,1 ± 0,0

Table 2(d)
pH evaluation of the barrier membranes.

Membrane	pH
Allogenic Collagen	7,3
Porcine Peritoneum	7,4
Porcine Dermis	7,3
Porcine Pericardium	7,4
Porcine Tendon	7,3
Porcine Bone Lamina	6,9
Equine Pericardium	7,3
Equine Bone Lamina	7,3
Bovine Tendon	7,3
Bovine Tendon + Hyaluronic Acid	7,3
Bovine Cross-linked Collagen	7,3
Porcine Cross-linked Collagen	7,3
Polycaprolactone	7,4
Polylactic-Glycolic Acid	7,4
Polytetrafluorethylene-dense	7,5

Exact p-values of each comparison are collected in Supplement document 1.

3.3. Absorption of membranes

All membranes, except Polycaprolactone and PTFE membranes, had a significant increase in weight over hydration time. Alloplastic membranes had the lowest absorption of all membranes tested (Table 2(c)). Porcine origin membranes had the greatest absorption, specially the Porcine cross-linked collagen membrane (Table 2(c)). Both laminas had very similar absorption rates and comparable to Allogenic Collagen (Table 2(c)). Bovine Tendon membranes had a greater absorption than other tendon membranes (Table 2(c)). Exact p-values of each comparison are collected in Supplement document 1. pH evaluation of the membranes showed that all membranes are in a physiological range between 6,9 and 7,5. Porcine Bone Lamina membrane had the lowest pH and PTFE-d membrane had the highest value (Table 2 (d)).

3.4. Wettability capacity of membranes

Contact angle of the membranes was determined after 4 and 30 s (Table 2(e)). Most membranes had a low contact angle after 30 s compared to the values obtained after 4 s. Rough surfaces in collagen membranes had a lower contact angle compared to the smooth surface in most membranes indicating a higher hydrophilicity. Alloplastic membranes had a high contact angle compared to collagen membranes. Exact p-values of each comparison are collected in Supplement document 1.

3.5. Physicochemical index

Radial graphs summarizing the data of all experiments was created (Fig. 2). Membranes with the same origin presented similar shapes. For example, alloplastic membranes or tendon membranes had a wing shape. Membranes that were less stiff (had a lower Young Modulus), had better results when normalizing to the less stiff membrane, whereas stiffer membranes (had a higher Young Modulus) had better results when normalized to the stiffest membrane (Table 3). Values of the physicochemical index range between 1 and 6 out of 10 (Table 3).

4. Discussion

To date no study has characterized the physical and chemical properties of barrier membranes from different origins. Although ideal barrier membranes properties have already been described (Caballe-Serrano et al., 2018; Rakhmatia et al., 2013), a complete characterization of each barrier membrane is still needed. The present study revealed that membranes from different origins have distinctive microstructure. Mechanical testing showed that bone lamina barrier membranes had a high tension withstand in their dry status, although when hydrated all membranes dropped their tension values, decreasing differences between membranes. Natural origin barrier membranes exhibited high absorption and wettability values, being lower in most alloplastic membranes and higher in natural origin barrier membranes, especially on their rough surface. The physicochemical index summarizing the previously reported data revealed similar results for the same tissue origin.

Previous studies compared the mechanical properties of barrier membranes and concluded that membranes should be chosen carefully depending on their origin and hydration status (Coic et al., 2010; Ortolani et al., 2015). All membranes presented similar results in terms of tension when hydrated for 4 min except for the bone laminas and allogenic collagen barrier membranes that exhibited higher tension rates. Barrier membranes became less stiff when hydrated, especially after 4 min, which coincides with a reduction in the tension. This is explained because the hydration of the collagen fibrils reconstitutes the native ultrastructure of collagen, increasing elastic capacity while reducing tensile strength (Zeugolis et al., 2009). Orientation of fibres could also play a role on the mechanical properties of the barrier membranes, as shown in studies that compare collagen constructs with different structure (Fratzl and Weinkamer, 2007).

The studied membranes reached a plateau after 6 min; this can be helpful in the clinical practice as some membranes are thought to be hydrated for long periods of time before placing them. Absorption evaluation revealed that in most cases porcine origin barrier membranes exhibited a major absorption, except for the Porcine Bone Lamina barrier membrane. The reason of this fact might be the origin of the membrane, being cortical bone. As previously described, cortical bone has only a 20% content of water, where soft tissues have between 64% and 79% of water (Mitchell et al., 1945; Techawiboonwong et al., 2008). Absorption values correlate with the values obtained from the wettability tests. Porous biomaterials tend to have a lower contact angle (Strong and Eaves, 2017); this was also seen in our study where porous natural membranes had a high wettability, especially after 30 s.

In the present study the authors confronted various limitations. First, the study offers indirect clinical evidence in terms of physicochemical properties; it might be challenging transferring these data to the clinical practice. Second, natural membranes show different behaviours ones from others; each natural membrane is unique because it derives from a different animal and different specific tissue. Third, exists the possibility that barrier membranes behave differently in

Table 2(e)
Wettability capacity measured with the sessile drop contact angle technique. Contact angle measured on the flat and rough surfaces at four and 30 s respectively. Dark blue colours were given to the higher wettability values (lower contact angles) whereas light blue colours were given to the lower wettability values (high contact angles). Four blue colours are used in total.

Membrane	Wettability (Contact Angle)			
	4s		30s	
	Smooth Mean ± SD	Rough Mean ± SD	Smooth Mean ± SD	Rough Mean ± SD
Allogenic Collagen	42,7 ± 81	43,3 ± 7,6	<1 ± 0	<1 ± 0
Porcine Peritoneum	<1 ± 0	<1 ± 0	<1 ± 0	<1 ± 0
Porcine Dermis	77 ± 13,9	91 ± 2,6	78,3 ± 8,6	<1 ± 0
Porcine Pericardium	95 ± 5,3	95 ± 5,3	<1 ± 0	<1 ± 0
Porcine Tendon	94 ± 6,2	72 ± 7,8	62,5 ± 4,7	<1 ± 0
Porcine Bone Lamina	73,7 ± 6,5	73,7 ± 6,5	64,7 ± 5,1	64,7 ± 5,1
Equine Pericardium	104,3 ± 3,8	35,7 ± 8,1	70,3 ± 3,2	<1 ± 0
Equine Bone Lamina	91 ± 3	91 ± 3	59 ± 25,2	59 ± 25,2
Bovine Tendon	110 ± 7	63,3 ± 15,3	103,7 ± 2,1	<1 ± 0
Bovine Tendon +Hyaluronic Acid	106,3 ± 2,9	103 ± 8,7	94,7 ± 18,1	95 ± 4,6
Bovine Cross-linked Collagen	50 ± 6	50 ± 6	<1 ± 0	<1 ± 0
Porcine Cross-linked Collagen	<1 ± 0	<1 ± 0	<1 ± 0	<1 ± 0
Polycaprolactone	102,3 ± 2,1	103 ± 2	99,3 ± 3,1	102,3 ± 2,3
Polylactic-Glycolic Acid	78,3 ± 2,17	134 ± 8,2	67,7 ± 4,9	132 ± 8,5
Polytetrafluorethylene-dense	106 ± 10,4	116 ± 1,7	105 ± 10,4	114,3 ± 2,3

biologic fluids or when they are degraded. Last, the vast amount of data presented in this manuscript may difficult the comprehension of each biomaterial and the differences between them.

Future studies should focus on studying hydrated barrier membranes as is closer to clinics, and chemically modified barrier membranes. Apart from the tissue origin it is possible to chemically modify barrier membranes (Caballe-Serrano et al., 2017; Duan et al., 2013; Teng et al., 2008) modifying their properties and increasing their degradation time. Adsorption of growth factors, liberation and later degradation of barrier membranes need to be studied for a better comprehension of membranes behaviour and possible applications. Furthermore, immune analysis of how barrier membranes behave and

Fig. 2. Summary of physicochemical properties of barrier membranes tested in radial graphs.

Table 3
Physicochemical index of the barrier membranes. First column shows the physicochemical index normalized to low stiffness, whereas the second column shows the physicochemical index normalized to high stiffness values, meaning that membranes that have a high stiffness (high young modulus) will have better values in the first column, and membranes with low stiffness (low young modulus) will have better values in the second column.

Membranes	< Stiffness	> Stiffness
Allogenic Collagen	5	4
Porcine Peritoneum	5	3
Porcine Dermis	3	2
Porcine Pericardium	2	2
Porcine Tendon	2	1
Porcine Bone Lamina	3	5
Equine Pericardium	4	4
Equine Bone Lamina	4	3
Bovine Tendon	3	2
Bovine Tendon + H.A.	2	1
Bovine Cross-Linked C.	3	3
Porcine Cross-Linked C.	6	5
Polycaprolactone	1	1
Polylactic-Glicolic Acid	1	1
PTFE-dense	1	1

degrade with human blood would bring valuable information. As future is leading to personalized medicine, upcoming studies could address the design and manufacture of customized barrier membranes with or without growth factors to answer specific patient needs.

5. Conclusions

The understanding of the physical and chemical properties of barrier membranes is essential for an optimal result. All membranes experienced a decrease in the mechanical properties once they were introduced into an aqueous solution. Different samples of the biomaterials presented similar pH values after the absorption test, in which porcine derived barrier membranes experienced an increased absorption capacity reaching a plateau in most cases after 4 min. Increased wettability values were obtained for rough surfaces. Different mechanical properties of barrier may affect the clinical characteristics of a biomaterial. More studies are needed for a further comprehension of the bone regeneration science.

Conflicts of interest

Authors deny any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmbbm.2019.04.053.

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