

# Mechanical qualification of collagen membranes used in dentistry

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## Abstract

**Aim.** The aim of this work is the qualification of commercially available collagen membranes in a comparative manner. The natural origin of collagen makes standardization difficult. Nevertheless, through dimensional and mechanical measures it is possible to mechanically qualify collagen membranes, and compare them.

**Methods.** Three commercially available collagen membranes used in Guided Bone Regeneration (GBR) and in Guided Tissue Regeneration (GTR) techniques, namely Bio-Gide, Collprotect and Jason, were chosen for the comparison. Quasi-static (tensile tests) and time-dependent (stress relaxation test) mechanical tests together with a functional test (tear test) were done to determine the responses of collagen membranes under different loading conditions.

**Results.** The tested membranes exhibited different behaviours, different deformability values and thickness, Jason being the thinnest and Bio-Gide the thickest. Similar differences were also observed in terms of surface density.

**Discussion.** Even though clinical observations were not within the aim of this study, our findings indicate that a better understanding of the correlation between mechanical properties and thickness could lead to a more rational design and use of these membranes in the face of specific clinical cases.

## Key words

- collagen
- membrane mechanical testing
- guided tissue regeneration
- guided bone regeneration

## INTRODUCTION

Collagen is an important biomaterial in medical applications thanks to its biological characteristics, such as bio- and cyto-compatibility and biodegradability [1]. It has been used in drug delivery systems [2], and in tissue engineering [3]. Collagen membranes are currently used as physical and biodegradable barriers in guided bone and tissue regeneration techniques [4-24] in dentistry.

The different collagen types [25-31] are characterized by considerable complexity and diversity in their structure, their linking variants, their assembly and their function. Great changes occur during the diverse manufacturing processes and the properties of extracted collagen can be modified to obtain forms which fulfill specific medical applications [32]. When it comes to GBR/GTR techniques, collagen is given the form of a membrane.

Membranes for GBR and GTR techniques must

feature certain characteristics, such as good tissue compatibility and cell occlusivity; they should serve as a physical barrier, be easily applicable for clinical use and have integrative properties [33]. An ideal barrier membrane is a biomaterial that serves as a barrier for an extended period and, after the intended bone regeneration is complete, becomes integrated with the surrounding soft tissue. Membranes have been assumed to take 4 weeks to achieve structural integrity in periodontal regeneration [33-36], whereas a longer period, *i.e.* up to 6 months, for bone tissue regeneration [33].

Several types of collagen membranes with varying biodegradability have been placed on the market [34] to satisfy tissue/bone regeneration requirements.

It is very difficult to compare collagen membranes just using the information reported in their datasheets as it refers to different processing conditions, sizes and properties, which in turn would render it impossible to

compare the clinical results of their application. As a matter of fact, even if the data reported were close to what is expected from them, they would never exactly match the need of the final user (clinician, dentist). As with any other engineering material, further experimentation is often required to fulfil the requirements for a specific application.

Nevertheless, through dimensional and mechanical measurements it is possible to compare different collagen membranes subject to the same test conditions. The aim of this case study is the mechanical qualification of commercially available natural collagen membranes used in guided tissue regeneration and in guided bone regeneration in a comparative manner.

The mechanical test performed on the membranes were examined in detail and the data ensuing from the related responses made comparison between membranes possible.

This is a complex case study: these membranes change their properties as they are resorbed and their selection should be critically evaluated before the specific clinical application. As a point of fact, the choice of materials and the design of implantable membranes influence the therapeutic potential and the associated clinical procedures [37].

## MATERIALS AND METHODS

Three commercially available collagen membranes of natural origin (porcine), Bio-Gide® (Geistlich Biomaterials, Baden-Baden, Germany), Collprotect® (Botiss Biomaterials, Italy) and Jason® (Botiss Biomaterials, Italy), were tested in different ways. Bio-Gide® has a bilayer structure with a compact outer layer and a porous inner layer of collagen fibre bundles, as described by the manufacturer. Collprotect® membrane originates from dermis and exhibits a degradation time of approximately 8-12 weeks and a slight stiffness. Its structure is a dense network of collagen bundles with pores for better vascularization, as reported in the manufacturer's datasheet. The same manufacturer describes Jason® membrane as designed and produced for dental tissue regeneration: originated from pericardium and with a long-lasting barrier effect of approximately 12-24 weeks. This membrane shows high adaptability during placement thanks to the fact that its structure consists of differently oriented collagen fibres, which provides multi-directional tear resistance.

The current study focuses on mechanical testing for membrane characterization rather than on the performance of commercial products. In view of the chosen approach, only one sample was used for each type of membrane. Before specimen extraction, a physical characterization of the three types of collagen membranes was carried out through the measurement of weight, surface density and thickness.

The nominal surface density was calculated as the ratio between the initial weight and its nominal surface area.

Thickness was measured with a digital calliper (Ruppec 239, resolution 0.001 mm, reference standard DIN 863/1). Membranes seem to be soft materials but actually not so much so as to need a no-contact measuring

system. In fact, measuring the thickness of extremely soft materials (such as flexible foams) with a calliper would result in strong errors because the measure depends on the applied pressure during the measurement. On the other side, these kind of materials would be also difficult to test under a tensile configuration because of clamping problems. That is not the case with membranes which can be efficiently measured by a calliper and tested after clamping. By using the calliper, a minimum pressure is applied over a large area and thickness measurement is significant and reliable.

Quasi-static (tensile test) and time-dependent (stress relaxation test) mechanical tests together with a functional test (tear test) were chosen to better identify the differences in response among membranes. For all the mechanical tests we used a universal testing machine (MTS Insight 5) with a load cell of 100 N.

After thickness measurements, six specimens (two for each test) were extracted from each membrane sample.

Tensile tests are typical tests performed for qualifying materials, especially if shaped in sheets or plates. During testing, a longitudinal strain is applied at constant rate along the main size of a rectangular or dog-bone shaped specimen, and the resulting force (or stress) is measured. Due to the small size of the membrane samples (about 15 x 20 mm<sup>2</sup>), the rectangular shape was chosen with a size of 2 x 15 mm<sup>2</sup>. Tests were performed with a gage length (*i.e.* distance between clamps) of 12 mm at a rate of 1 mm/min up to a maximum displacement of 10 mm until specimen failure. Several data can be extracted from tensile tests such as the tensile modulus (*i.e.* stiffness per unit of volume), the maximum elongation, and the strength (*i.e.* maximum attainable force per unit of area).

Material failure under tensile load is very rare in the actual working conditions of the materials [38, 39].

Failure of these collagen membranes can reasonably occur because of tearing during placement by the dentist or as a result of further loading conditions. There is no way to infer the tearing behaviour of a sample starting from the analysis of a tensile test.

Tear tests give a better comparison among different materials as they provide information on the energy or force required to propagate a tear through the material. Specimens were cut with a nominal area of 5 x 15 mm<sup>2</sup>, and the test was initiated with a 7 mm long central cut. Tear propagation was monitored as a function of the vertical displacement at the constant rate of 1 mm/min, up to a maximum displacement of 10 mm.

In the end, stress relaxation tests were performed. The response of organic materials to load depends on the rate the load is applied at and on test duration. Being visco-elastic materials, the load initially applied in an elastic range (therefore a reversible load) during time converts in viscous flow. To measure this effect, a load can be applied and its change over time measured in stress relaxation tests. Specimens with the same size as those used for tensile tests (2 x 15 mm<sup>2</sup>) were tested in tensile configuration imposing a stress value of 2 MPa for Collprotect and Jason membranes, and 1 MPa for the softer Bio-Gide. Stress relaxation was evaluated over a time span of 5 min.

## RESULTS

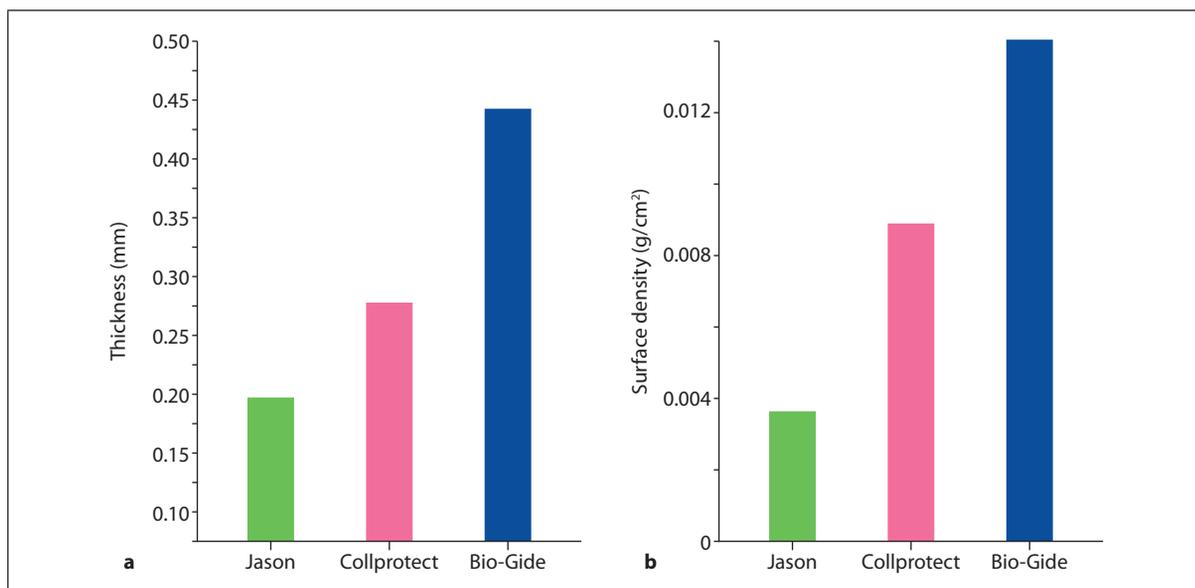
Figure 1a reports results from thickness measurements. For each membrane the nominal surface density was calculated by the ratio between the sample weight and its area (Figure 1b). Thickness and surface density data are also reported in Table 1.

Figure 2 shows tensile tests curves in terms of engineering stress-strain, and the experimental tensile test configuration. Load and displacements were measured during the test, but these data were converted into engineering stresses and strains to make the comparison between specimens possible: the engineering stress is extracted by dividing the tensile load by the initial section; the engineering strain is the displacement divided by the initial gage length. Tensile curves seem to be particularly smooth despite of the inhomogeneous nature of this kind of materials. In particular, a bilinear stage is generally present in the first part of the curves up to a maximum at which failure occurs. After failure, a residual strength is visible because part of the membrane continues to stretch. Stretching is probably one of the reasons for the initial bilinear stage. An elastic modulus can be extracted from the slope of the first linear trend. The elastic modulus is the evaluation of the ma-

terial stiffness per unit of volume and is an easy way to compare materials, membranes included. Extracting an elastic modulus from this kind of curves could be affected by the presence of plastic (*i.e.* non reversible) strains; nevertheless, the validity of the comparison (shown in Figure 3a) still holds. Another interesting comparison can be provided by the maximum stress or the related maximum strain (elongation) which are plotted together in Figure 3b. This kind of graph allows us to immediately identify the failure behaviour of materials or their possible data scattering.

Figure 4a shows the results from tear tests. Curves are reported in terms of tear load along the displacement. All the curves are characterized by several peaks which are typical for this kind of fibrous materials. A single tear force value can be extracted by averaging the tear load in a fixed displacement range. Tear force has been calculated in the displacement range between 2 and 6 mm. Despite the scattering of the tear test curves, final tear forces seem to be reliable enough for each single membrane.

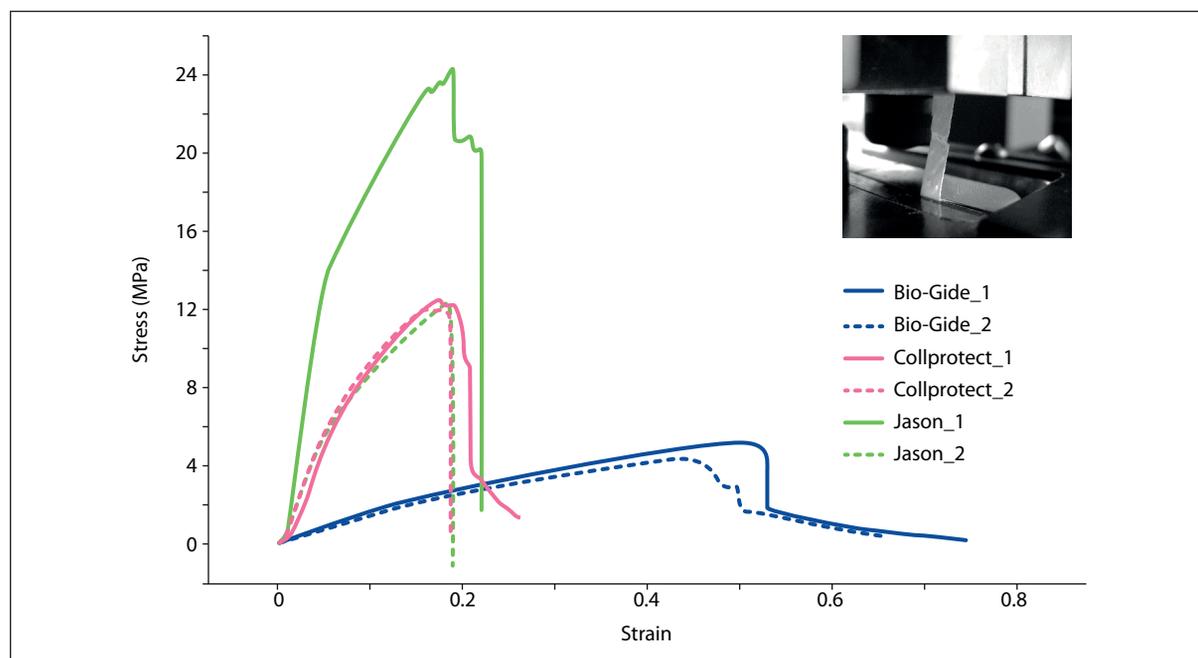
Figure 4b reports stress relaxation curves after normalization with the maximum stress (*i.e.* the applied tensile stress). With a stress relaxation test we measure



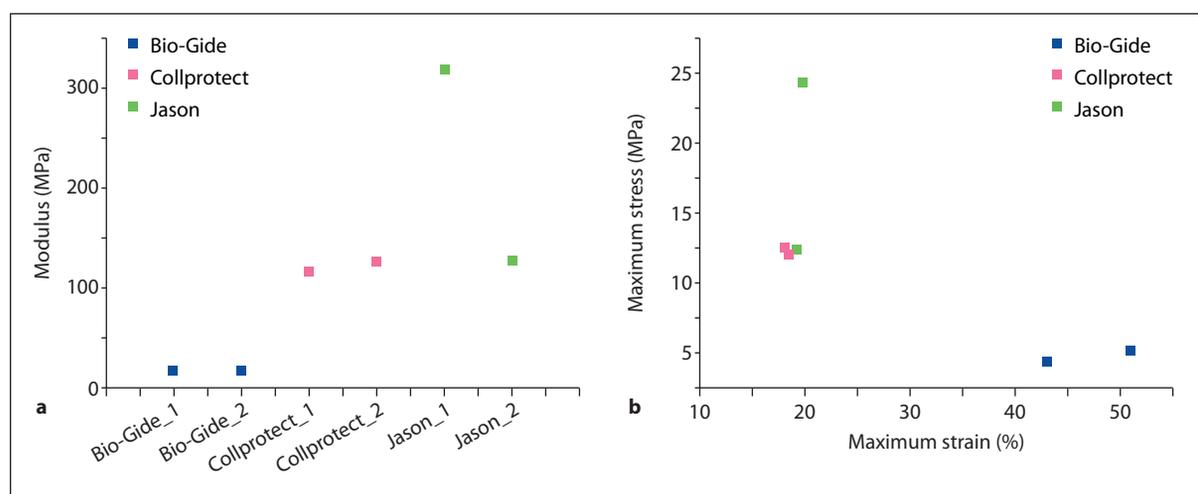
**Figure 1**  
Thickness (a) and surface density (b) of the collagen membranes.

**Table 1**  
Average physical and mechanical data for all the tested membranes

	Bio-Gide	Collprotect	Jason
Thickness (mm)	0.44	0.28	0.20
Surface density (g/m <sup>2</sup> )	140	90	40
Tear load (N)	1.84	1.15	0.74
Elastic modulus (MPa)	15.7	158.5	178.9
Maximum tensile stress (MPa)	4.8	13.1	13.0
Maximum tensile strain (%)	46.8	16.3	17.9
Stress relaxation (%)	26.4	16.9	22.1



**Figure 2**  
Tensile test curves.



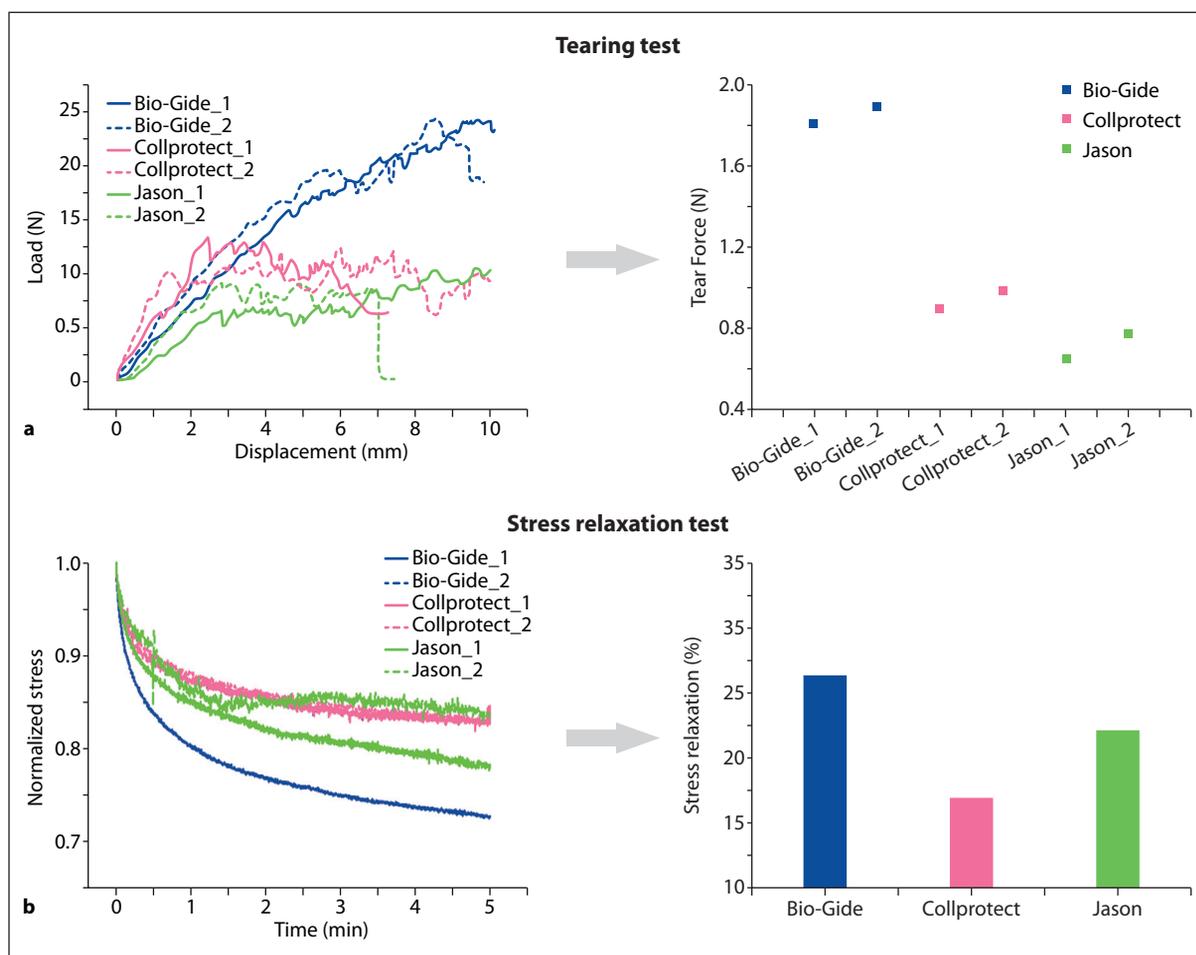
**Figure 3**  
Elastic modulus (a) and ultimate properties (b) extracted from tensile curves.

the time-dependent response of materials under constant loading conditions. The load is applied in terms of fixed displacement and tends to decrease with time because of material mobility. As a consequence, stress relaxation curves start from an initial stress down to zero or a plateau value. Normalization was needed in order to be able to compare materials tested at different stress values (2 MPa for Jason and Collprotect, 1 MPa for Bio-Gide). In optimal conditions, stress relaxation tests have to be performed in elastic range. On the other side, the higher the initial stress, the better the measurement, therefore different initial stress values were chosen for the membranes because of the differences in tensile tests (Figure 2). Five minutes was a sufficient time period to observe a strong stress reduc-

tion. In order to make the comparison between the materials clearer, again a single stress relaxation value was extracted from each curve. A good solution is extracting the "stress relaxation" in terms of percentage reduction of the initial tensile stress (*i.e.* the final values of curves in Figure 4b). The stress relaxation value is shown in terms of average value for each membrane, and is also reported in Table 1 together with all the main results from tensile and tearing tests.

## DISCUSSION

Measuring the thickness of membranes is rather simple but, owing to the tissue-like nature of the samples, it may become a quite complex issue when it comes to interpretation of results. However, other measurements



**Figure 4**  
Results from tearing (a) and stress relaxation tests (b).

were made (by mechanical tests) in this study and a correct comparison is possible from a global analysis of all the results. From another point of view, thickness measurement is fundamental for results normalization: for example, to extract tensile stresses from measured tensile forces.

In analogy with thickness (*Figure 1a*), strong differences were found also in terms of surface density (*Figure 1b*): by increasing the former, the latter increases as well. As expected, a higher thickness results in more material per surface unit.

High-performance materials should show high strength (*i.e.* high maximum tensile stress) at high elongation values. Typically, increasing strength results in decreasing elongation: the preference for one characteristic or the other will depend on the technical application. Typically applied to engineering materials, especially when in the shape of sheets or plates, tensile tests (*Figure 2*) have been used as a first approach to get information about the mechanical performance of the membranes [40]. Performing tensile tests is easy and fast, and related results are often easy to manage.

In *Figure 3a* the elastic modulus shows a good result (low scattering data) for Bio-Gide and Collprotect but

not for Jason, the stiffest one. Bio-Gide is the softest membrane despite of its higher surface density. In any case, the mechanical properties of fibrous materials mainly depend on the bonding among fibres rather than on their amount [40]. This aspect could be also responsible for the high scattering of data for Jason which can also be seen in *Figure 3b*. Jason exhibited on average the highest strength, but the two tested specimens behaved quite differently: the maximum tensile stress of one specimen was twice as high as the other, but the related maximum strain (elongation) was comparable. Instead, Collprotect specimens behaved in a very similar way with a minimal difference in terms of elastic modulus, maximum tensile stress and stress relaxation. *Figure 3b* also confirms the lower performance of Bio-Gide with an acceptable scattering for all the extracted tensile properties.

It is remarkable that tensile curves appear very smooth (*Figure 2*) while the extracted data show a significant scattering (at least for Jason); conversely tear test curves are very scattered (*Figure 4a*) but the extracted tear force data are not scattered for all the membranes. Although the number of performed tests is not enough for a statistical analysis and only qualitative

observations can be made, tear test allows for a comparison between the membranes in spite of their complex structure. The stiffest material (Jason) under tensile load shows the lowest tear forces, whereas the most ductile one (Bio-Gide) shows the highest tear forces.

As with tensile properties, Collprotect is in the middle again. This confirms the ability of the adopted testing procedure in representing a significant view of the mechanical behaviour of this class of materials. In fact, rigid fibrous materials could be expected to exhibit lower tear forces, since the applied forces easily tend to break the fibres instead of stretching them.

In Bio-Gide, thanks to its deformability, fibers are re-arranged during tearing and the ensuing orientation increases tear strength. Differently from tensile testing, during tear tests materials are subjected to shear stresses and material mobility can obstruct crack propagation [41, 42]. As a consequence, the energy or force, required to propagate a tear through the material, is used to re-arrange the material rather than break it.

Collprotect is a membrane with a medium level of elasticity and mobility; in fact, its behaviour is intermediate between Jason and Bio-Gide. In view of the results from tensile and tear tests, stresses should be expected to relax more easily in soft Bio-Gide than in stiff Jason.

Even if only two tests were made for each membrane, a good repeatability has been found for stress relaxation curves (*Figure 4b*) in analogy with tensile test curves (*Figure 2*): this similarity probably depends on the used tensile-load configuration. Average stress reduction after 5 minutes partially confirms the expectations about the difference between Jason and Bio-Gide membranes. In fact stress relaxation in Bio-Gide was over 26% whereas Jason membrane was about 22%. Despite of results from tensile and tear tests, in the case of stress relaxation, Collprotect is not in between the other two membranes: it exhibits the lowest stress relaxation value. A possible explanation could be that the data scattering already observed for Jason could have played a negative role in defining its behaviour during stress relaxation. On the other hand, this result is important to understand that complex correlations exist between mechanical performances in this kind of materials, and mechanical testing is the only way to go deeper into these aspects. In order to understand the

reason for this mismatch, stress relaxation tests should be repeated with more samples at different initial stress values, and with stress reductions after different time periods. Anyway, this investigation is not within the aim of this study which focuses on the need of mechanically qualifying this class of materials rather than exactly identifying their performances.

## CONCLUSION

All the membranes used for the testing, though meant for similar applications, behave in a different way.

Complementing data from performed mechanical and functional tests with data already present in the scientific literature about the biological properties of membranes used in dentistry and, above all, complementing them with the experience and competence of each single dentist should result in a better understanding of the behaviour of these materials during their placement and working conditions. In fact, beside the typical biological and clinical data, data from mechanical and functional tests on collagen membranes could be appropriate to predict their effect in specific clinical cases.

The results hereby presented are not intended to help users choose one kind or another for a given clinical case. The idea is to provide clinicians with as much data as possible so as to help them in a successful comparison. Further investigation of the correlation between mechanical properties and thickness is needed for a more rational design and use of these membranes.

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## Conflict of interest statement

No conflict of interest.

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