STUDY REGARDING THE ROLE OF BARRIER MEMBRANES IN GUIDED BONE REGENERATION TECHNIQUES

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ABSTRACT

Aim of the study Resorbable barrier membranes have emerged as a crucial component of GBR procedures, facilitating the controlled healing process and promoting bone regeneration. This review aims to explore the roles, comparison and benefits of resorbable barrier membranes in guided bone regeneration. **Material and methods** In this scientific paper, FTIR (Fourier Transform Infrared Spectroscopy) and SEM (Scanning Electron Microscopy) analyzes were performed for four sets of collagen-based dental membranes and pericardium. The tests were conducted in the laboratories of the Faculty Materials Science and Engineering, Politehnica University Bucharest. **Results** The clinical applications and outcomes of resorbable barrier membranes in guided bone regeneration are extensively discussed. Studies have demonstrated the effectiveness of these membranes in enhancing bone regeneration and improving the long-term success of dental implant placements. The influence of the material from which the resorbable membranes are made on the contact angle was highlighted, even if they have the same duration of resorbability. The influence of the material from which the resorbable membranes are made on the contact angle was highlighted, even if they have the same duration of resorbability (P2, P3, P4). **Conclusions** Resorbable barrier membranes play a crucial role in guided bone regeneration by creating a protected environment, facilitating osteogenic cell migration, and promoting bone formation. With careful consideration of their properties and proper surgical techniques, resorbable barrier membranes offer a valuable tool in enhancing bone regeneration and achieving successful oral and maxillofacial reconstruction procedures. Further research and advancements in membrane design and clinical protocols will continue to refine and optimize their application in guided bone regeneration.

Key words: barrier membranes, guided bone regeneration, scanning electron microscopy

INTRODUCTION

Barrier membranes have an important role in Guided Bone Regeneration, avoiding the invasion of soft tissues in bone defect and creating the space to sustain the new bone growth in normal parameters.

Guided tissue regeneration techniques are widely used in the reconstruction of the muco-osseous support in oral complex rehabilitation. The reconstruction of the alveolar bone with severe resorption is requested to allow proper implants positioning and long-term outcome of the implant-prosthetic therapy (Tolstunov et al, 2019; Urban & Monje, 2019; Wessing et al, 2018). Biomaterials used in these techniques are various categories of bone grafts (autogenous bone, xenografts, alloplastic grafts) combined with barrier membranes.

The placement of a barrier membrane to avoid the penetration of the non-osteogenic components (epithelial and connective tissue

cells) in the bone compartment and interfering with bone regeneration processes is the primary principle of guided bone regeneration technique (Sasaki et al, 2021; Elgali et al, 2017). The barrier membranes used in the guided tissues regeneration techniques are bioactively components while the molecular and cellular activities inside the membrane are linked to the stimulation of the alveolar bone regeneration (Sasaki et al, 2021; Omar et al, 2019). The regeneration of bone deffect in guided bone regeneration techniques last 4-6 weeks for periodontal tissues and 16-24 weeks for alveolar bone. Most studies reported similar outcomes regarding the level of vertical and lateral bone gain in guided bone regeneration techniques performed with both resorbable and non-resorbable membranes (Patil et al, 2023). In this context, clinicians must understand the physicochemical, mecanical and biologic properties of different materials in relation to their tissue origin (Caballé-Serrano et al, 2019). However, in the interpretation of the literature data provided by studies focused on the role of membranes in the guided tissue regeneration techniques, we must consider the relation between clinical outcomes and the clinician performance and experience, as well as medical history, periodontal history, oral hygiene or smoking (Zhang et al, 2022).

The properties of an ideal membrane material are as follows (Naung et al, 2019):

1. Biocompatibility- ability to be effective without undesired interactions between human organism and material ;
2. Cellular exclusion- set-up of barrier against penetration of epithelial cells ;
3. Space creation and maintenance inside bone deffect- mechanical and structural properties of membrane allow it to resist against forces and prevent the collapse of the adjacent soft tissues ;
4. Selective permeability- allow fluids exchanges;
5. Biologic activity- resistance to microbial action, good support for antibiotics or growth factors;
6. Satisfactory handling properties.

The barrier membranes are divided in two categories: resorbable collagen membranes and non-resorbable membranes.

The resorbable membranes used in the guided bone regeneration techniques are natural and artificial polymer membranes (Zhang et al, 2022). These membranes are divided, related to their origin, as follows:

• natural polymers, represented by collagen;

• synthetic polymers represented by aliphatic polyesters (eg poly (lactic acid) (PLA), poly (polyglycolic acid) (PGA), poly (ε-caprolactone) (PCL).

Due to limits of the non-resorbable membranes, collagen membranes are still the most frequent used in the guided bone regeneration techniques due to scientific background and extensive clinical validation (Ren et al, 2022).

The great advantages of the resorbable collagen membranes are hydrophilicity and easy of handling. Other benefits include (Zhang et al, 2022; Allaudin et al.,2022):

- low cost:
- guide for soft tissue healing;
- increased biocompatibility;
- protection of the immature bone tissue from soft tissue invasion;
- impermeable to cells, but permeable to nutrients;
- resorption capacity through enzymatic degradation without causing tissue irritation.

The limits of the native collagen membranes are the inability to maintain the proper space needed to cover severe bone

defects (Soldatos et al, 2017; Turri etal, 2021).

The modified collagen membranes by crosslinking can provide better results in soft tissues and bone regeneration, due to better mechanical strength and degradation cycle (Ren et al, 2022). Despite excellent biocompatibility, unpredictable degradation profile of the collagen membranes can reduce the effectiveness of the guided bone regeneration techniques (Bozkurt et al, 2014). The modification of collagen membranes is necessary and effective to achieve better clinical tissue regeneration, generally through cross-linking and the transport of bioactive molecules. The initial hypothesis of the cross-linking process is to improve the mechanical strength and degradation cycle of collagen membranes. and thus to influence the clinical outcome of collagen membranes. The identification of the chemical components of collagen (qualitative analysis) and finding out their amount (quantitative analysis) can be done by SEM and FTIR microscopy (Riaz T et al, 2018; Karen et al, 2019).

MATERIAL AND METHODS

In this scientific paper, FTIR (Fourier Transform Infrared Spectroscopy) and SEM (Scanning Electron Microscopy) analyzes were performed for four sets of collagen and pericardial dental membranes.

The four membranes are shown in the figures below (Fig. 1.1, Fig. 1.2, Fig. 1.3, Fig. 1.4).

Figure 1.1 Collagen membrane - bovine origin

The resulting signal in the detector represents the molecular fingerprint of the sample and each molecule or chemical structure generates a unique spectral fingerprint, making it an excellent tool for chemical identification.

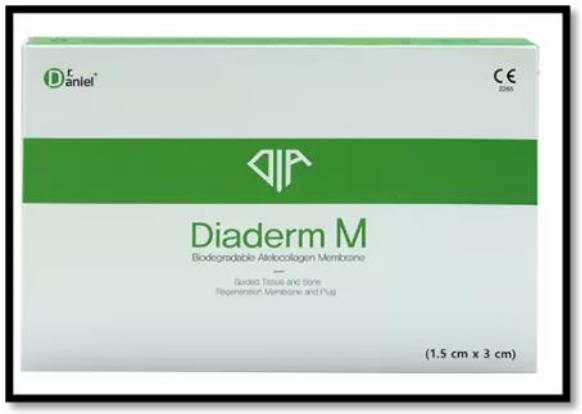


Figure 1.2 Collagen membrane - porcine origin

The abbreviation FTIR (FourierTransform InfraRed) stands for "Fourier Transform Infrared" and is the most common form of infrared spectroscopy. All infrared spectroscopies work on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed.

The advantages of the method are as follows: it does not destroy the sample, it is significantly faster than older techniques,

being much more sensitive and precise.

Figure 1.3 Pericardial membrane - equine origin

FTIR spectroscopy is an established quality control technique for evaluating materials produced in various industries and often serves as the first step in the materials analysis process. The tested samples are presented in Table 1.

Table 1. Coding of experimental samples

Probe	Material	Producer	Resorbability interval
P1	Collagen membrane - bovine origin	Mucoderm	6-9 months
P2	Collagen membrane - porcine origin	Diaderm M	3 months
P3	Pericardial membrane – equine origin	Proguard Lyo	3 months
P4	Pericardial membrane – equine origin	Heart	3-4 months

This technique is useful for analyzing the chemical composition of particles between 10 and 50 microns in size. Identifying the chemical structure of collagen is very important in the study of making a collagen-based dental membrane, and Fourier transform infrared (FTIR) is an advantageous technique to study their chemical structure.

Figure 1.4 Pericardial membrane – equine origin

Scanning electron microscopy (ScanningElectronMicroscopy) is a method by which the surface of a material can be visualized by scanning an electron beam on it, but also for the morphological analysis of micro- or nanostructures.

At the same time, this method serves to enlarge a certain region in the sample, using a high-energy focused electron beam.

The sample is under vacuum to ensure that the electron beam remains focused and does not interact with particles in the air. When the electron beam hits the sample, it releases secondary electrons from the sample to provide an image based on the surface topography.

The two most commonly used detectors are the Secondary Electron Detector (SED) and the Backscattered Electron Detector (ESB). The electrons interact with the detector to create an image, viewed electronically. Microstructural images of the morphological details are provided, which contribute to providing some results in the qualitative analysis.

The samples to be tested can be homogeneous or inhomogeneous three-dimensional solid

materials of different shapes or types: thin, micro- or nanostructured films, threads or powders.

Wettability, defined as the interaction of the solid surface in contact with a liquid medium (distilled water), is one of the most important characteristics of the surface of biomaterials.

At the same time, it represents the property of a material surface to come into direct contact with water molecules through hydrogen bonds.

Water molecules can penetrate through the pores of the material and completely wet the surface.

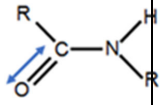
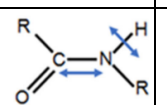
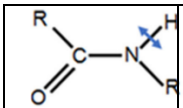
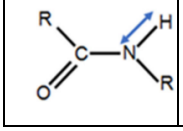
Most natural compounds (natural polymers, proteins, polysaccharides, etc.) are hydrophilic. Hydrophilic coatings are very effective and maintain the effect on a surface for a very long time.

The contact angle is determined by aligning the tangent of the drop profile with the surface at the point of contact. Under the same conditions, the measurements are repeated and the results are presented as an average value.

RESULTS AND DISCUSSIONS

The characteristic bands of collagen are similar to those of other proteins. The IR spectrum of collagen shows bands for amide I, amide II, amide III, respectively amide A and amide B. The functional groups and the wave numbers at which they appear in the FTIR spectrum are presented in Table 2.

Table 2. Functional chemical groups present in the chemical structure of collagen

Structure Amide	Type	Chemical bonds	Wave number (cm^{-1})
	I	C=O	1620 < ν <1800
	II	N-H + C-N	1590< ν <1650
	III	N-H	1200< ν <1400
	A	N–H + O-H	3300< ν <3400

In the FTIR spectra obtained on the investigated samples, the bands characteristic of the chemical structure of collagen were identified Table 3.

Table 3. Identification of bands characteristic of the chemical structure of collagen.

Wave number (cm^{-1})	Chemical bond	Functional group
~3290	υ(N–H) +υ(O-H)	Amide A
~3070	υ(C-H)	Amide B
~1634	υ(C=O)	Amide I
1540-1547	δ (N-H) + υ (C-N)	Amide II
~1236	υ(N-H)	Amide III
~2930	υ(C-H)	Metilen, -CH_2
~1448	δ(C-H)	Metilen, -CH_2

The characteristic bands of collagen are similar to those of other proteins. The IR spectrum of collagen shows bands for amide I (~1634 cm-1), II (1540-1547 cm-1) and III (~1236 cm-1), respectively amide A (~3290 cm-1) and amide B (~ 3070 cm-1).

The amide I band is sensitive to conformational changes of the compound of which it is a part and is frequently used to highlight the secondary structure of proteins.

The amide I band appears in the collagen structure due to the stretching vibrations of the carbonyl (C=O) group.

The amide II band is due to the strong (N-H) bending vibration coupled with the (C-N) stretching vibration, and the amide III band

appears due to the (N-H) group bending vibration.

Amide A band is due to (N-H), and (O-H) stretching vibration and amide B band is due to (C-H) stretching vibration.

The graphic representation of the FTIR spectra for each sample is in Figure 2. Through the obtained graphs we were able to establish and confirm the presence of functional groups specific to collagen, at their specific wave number.

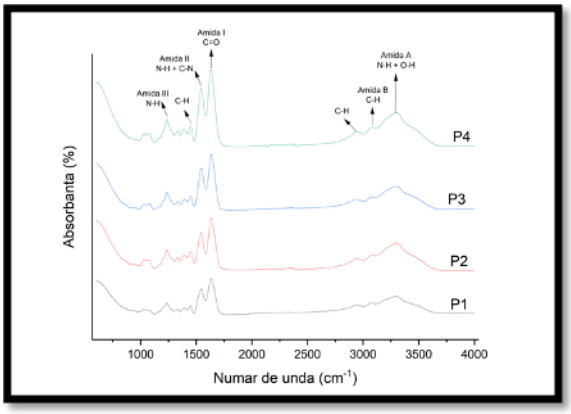


Figure 2 Graphical representation of FTIR spectra for each sample.

For each individual sample, SEM microscopy was carried out, at 100x magnification, CBS detector to be able to visualize the surface of the material in depth, along with the observation of morphological details, which contribute to providing results in the qualitative analysis.

Microscopy images correspond to figures 3, figures 4, figures 5, figures 6.

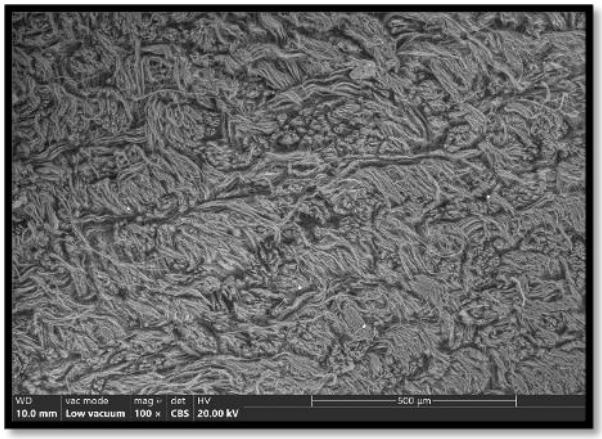


Fig 3. SEM image for the collagen membrane - bovine origin - P1

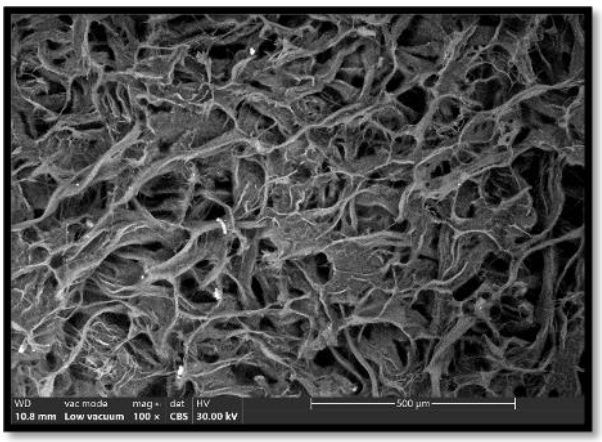


Fig 4. SEM image for the collagen membrane - porcine origin - P2

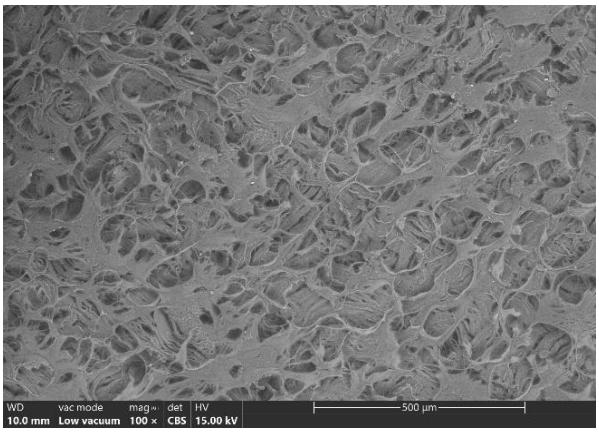


Fig 5. SEM image for the pericardial membrane - equine origin - P3

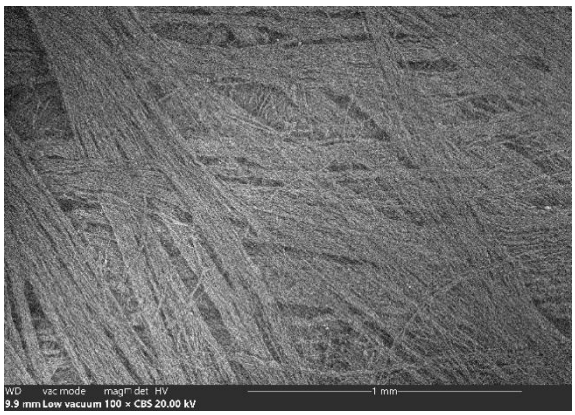


Fig 6. SEM image for pericardial membrane - equine origin - P4

The P2 membrane from collagen extracted from porcine dermis has a porous structure with a conical pore shape.

These are the most suitable for osseo-integration processes, because due to the shape of the pores, preosteoblasts can easily proliferate through the membrane structure, thus favoring the welding of the implant into the bone by creating new biological structures.

In comparison with P2, samples P1, P2 and P3 present a fibrillar structure, and for this reason it is given a much more pronounced mechanical resistance than the collagen membrane extracted from porcine dermis (P2).

Due to the absence of an interconnected porosity in the membrane structure, despite the more pronounced mechanical properties, they do not have the same performance in the field of osseointegration, preosteoblasts not being able to profile through the membrane and thus favor the welding of the bone to the graft.

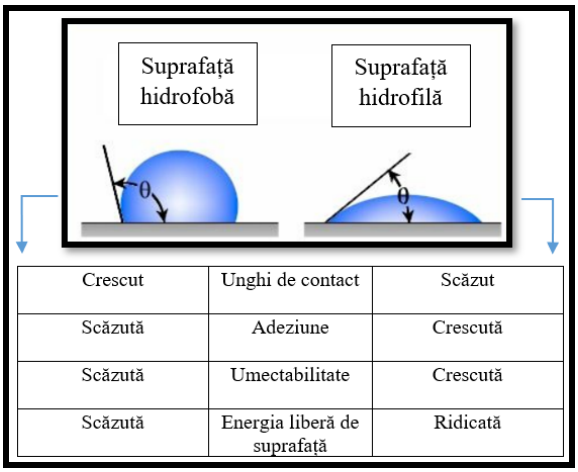
The contact angle is defined as the angle formed at the intersection of the liquid-solid interface with the liquid-vapor interface (this is obtained geometrically by drawing a tangent from the contact point to the liquid-vapor interface in the drop profile).

Determining the wettability of the collagen-based membranes under study is important for evaluating the biological response of the membrane after implantation.

In the case of adequate hydrophilicity, cell adhesion and proliferation increase, and osteogenesis occurs at the interface between the biological environment and the material.

A low value of the contact angle ($\theta < 90°$) defines a hydrophilic surface favorable for the absorption of molecules from biological fluids.The physico-chemical property of the wettability studied surfaces, along with the surface topography, are essential in achieving/optimizing cell adhesion and proliferation.The behaviors are presented in Table 4.

Table 4. Hydrophobic and hydrophilic surface



Crescut	Unghi de contact	Scăzut
Scăzută	Adeziune	Crescută
Scăzută	Umectabilitate	Crescută
Scăzută	Energia liberă de suprafață	Ridicată

The contact angle analysis was performed for each sample, thus establishing the wettability parameters (time, contact angle) for the surfaces of the studied membranes. The evidence of this test is represented in figures 7.1, figures 7.2, figures 7.3, figures 7.4.

RESULTS AND DISCUSSIONS

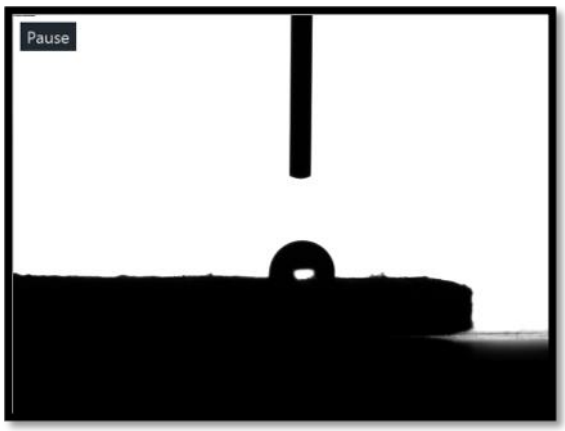


Fig 7.1 Determination of the contact angle (P 1) 87.23°

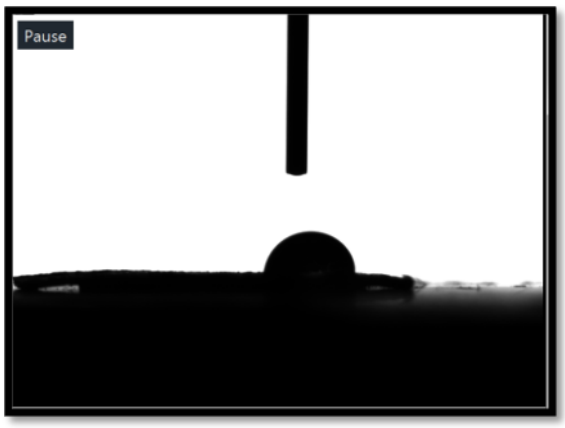


Fig 7.2 Determination of the contact angle (P 2) 83.29°

Fig 7.3 Determination of the contact angle (P 3) 47.21°

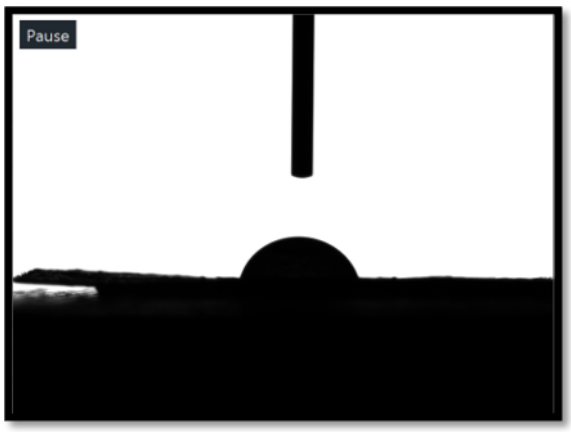


Fig 7.4 Determination of the contact angle (P 4) 48.83°

RESULTS AND DISCUSSIONS

The mechanical and physicochemical properties of the resorbable membranes varies widely between commercial products. The importance of *in vitro* studies is highlighted by the influence of these properties on the clinical handling and long-term implant survival and success. *In vitro* research groups investigated various properties such as chemical composition, tensile strength, modulus Young, wettability, roughness, density, thickness and porosity (Ren et al, 2022; Caballé-Serrano et al, 2019; An et al, 2018), but only few research groups investigated the surface microarchitecture of the resorbable membranes (Tai et al, 2023; Mauad de Abreu et al, 2020). Mostly, studies on surface topography of the resorbable barrier membranes were performed by using SEM technique. These studies compared cross-linked collagen and non-linked collagen membranes (Mauad de Abreu et al, 2020), various non-cross linked porcine-derived collagen membranes (Tai et al, 2023). While cross-linked collagen increases bioabsorption

time and structural stability of the resorbable membranes, non-cross-linked membranes have higher porosity, excellent hemostatic properties, and increased cell colonization (Abreu et al, 2020). Porcine-derived collagen membranes from different sources and manufactured by different processes had similar fibril distribution as well as the similar diameters of collagen fibrils, but different deformation grades of collagen during manufacturing process due to differences between D-periodicity of the fibrillar collagen (Tai et al, 2023). A previous study compared collagen with expanded polytetrafluoroethylene (ePTFE) and found that the latter inhibits gingival fibroblast synthesis, the former enhances cell proliferation (Quteish et al,1991). Another study performed an enzyme-linked immunosorbent assay and found no specific immunoreaction against collagen (Schlegel et al.1997). Furthermore, collagen matrix (Mucograft) infused with recombinant human platelet- derived growth factor BB (rhPDGF-BB) effectively increases gingival thickness prior to anterior implant prosthesis fixation (Simion at al,2012). A collagen membrane infused with rhPDGF-BB was placed over the implant, and sufficient healing time was allowed prior to tissue thickness measurement)Simion at al,2012]. The porcine-derived collagen bioactive membrane CelGro^{TM} (Orthocell Ltd., Murdoch, Australia) was developed for GBR in dental and orthopedic applications (Hassan et al,2017). CelGro^{TM} promotes vascularization (Chan et al,2016), induces cellular recruitment (Turri et al,2016) and upregulates pro-osteogenic factors at the implant site (Taguchi et al, 2005). Compared to with the commercially available collagen membrane Bio-Gide®, CelGro^{TM} shows much better cortical alignment and lower poros- ity at the defect interface. Celgro^{TM} can restore bone defects without complications or adverse events.

Collagen membranes can modulate the osteoimmune response of macrophages. Chen et al. modified a collagen membrane by coating it with a nanometer bioactive glass (hardysonite) through pulsed laser deposition for GBR and evaluated its ability to enhance osteogenesis through osteoimmunomodulation (Chen et al, 2018). They found that the modified collagen membrane can enhance the osteogenic differentiation of bone-marrow-derived mesenchy- mal stem cells, suggesting that collagen membranes with nanometer-sized hardysonite coating are promising for GBR applications. In addition, Annen et al. developed a colla- gen membrane with prolonged resorption time to overcome early resorption limitation. However, the results showed significantly higher membrane exposure in the new collagen membrane than in the native collagen membrane (Annen et al, 2001).

The magnesium membrane has been proven to have all of the necessary requirements for an optimal regenerative outcome from both a mechanical and biological perspective.

The *in vivo* performance study demonstrated that the magnesium membrane has a comparable healing response and tissue regeneration to that of a resorbable collagen membrane. (Rider et al,2022).

Research groups highlights the need for more *in vitro* studies regarding adsorption, integration capacity and rate of degradation of the resorbable barrier membranes for better understanding of their clinical and biological behavior (Ren et al, 2022; Sbricoli et al, 2020; Caballé-Serrano et al, 2019).

In our study, the influence of the material from which the resorbable membranes are made on the contact angle was highlighted, even if they have the same duration of resorbability (P2, P3, P4).

Due to the difference between the porous structure (P2 membrane) and the fibrillar structure (P1 membrane) it has been shown that the design and processing of biomaterials influence resorbability.

The comparisons and interpretation of the literature data is difficult due to differences in important parameters, such as the alveolar bone location, treatment protocol, treatment duration and postoperative check-up, as well as different criteria for evaluation (Zhang et al, 2022).

CONCLUSIONS

1. The influence of the material from which the resorbable membranes are made on the contact angle was highlighted, even if they have the same duration of resorbability.
2. Due to the difference between the porous structure (P2 membrane) and the fibrillar structure (P1 membrane), it has been shown that the design and processing of biomaterials influence resorbability.
3. Resorbable barrier membranes serve as physical barriers, preventing soft tissue ingrowth while allowing the migration of osteogenic cells from adjacent bone into the defect area.
4. The membranes help to stabilize the blood clot, maintain a space for undisturbed bone regeneration, and facilitate the recruitment of mesenchymal stem cells and progenitor cells.
5. Factors such as membrane degradation rate, exposure to bacterial contamination, and immune responses may affect the regenerative outcomes.
6. The resorbable barrier membranes play a crucial role in guided bone regeneration by creating a protected environment, facilitating osteogenic cell migration, and promoting bone formation.

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