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# PRODUCTION OF BARRIER MEMBRANE FOR ZEIN-GUIDED BONE REGENERATION

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**Abstract**: Guided Bone Regeneration (ROG) consists of a treatment to achieve bone regeneration, after tooth extraction, to recover the bone and alveolar ridge, making implant placement possible. Treatment with ROG is based on the application of a barrier membrane to prevent cellular invasion of soft tissue into the alveolar cavity, preventing bone defects. From the zein protein found in the corn grain, a barrier membrane for ROG was made. The zein was extracted using a simple methodology of extraction with isopropyl solvent under heating and agitation, purified and made in barrier membranes, which were characterized in terms of their morphological, mechanical and biodegradation properties, in terms of their barrier capacity against cell invasion and cytotoxicity assays. The protein extraction was carried out successfully, and its yield is satisfactory for use in all the assays present in this work.
**Keywords:** Bone regeneration, zein, maize protein, mechanical properties, resorbable membranes, cell invasion, cytotoxicity.

## INTRODUCTION

The present study developed a membrane with a plant-derived protein known as zein, with biodegradable possibilities for guided bone regeneration (RGO), with its particularities discussed throughout the work. For a better understanding of the subject, some concepts and definitions about protein and RGO will be discussed.

Plant-derived proteins are of great interest because they are renewable and biodegradable, as they offer environmental benefits over petroleum-based materials (BISHARAT et al., 2017).

The use of zein for industry is still little known, this protein is found in the endosperm of corn, with a high content of non-polar amino acids, thus allowing a hydrophobic molecular structure, solubilizing itself in alcohol, allowing its extraction (SHUKLA & CHERYAN, 2001 apud PAPALIA; LONDERO, 2015).

As well as for Bisharat et al. (2017) and also by Papalia and Londero (2015), zein represents 60% of the total of all proteins found in corn grain, characterized by its similarity in molecular mass, solubility in solvents and a structure of four subclasses: alpha, beta, gamma and delta, alpha-zein being the most abundant. A property of zein that is interesting for application in the field of biomaterials is its ability to form films, that is, a filmogenic material. This property expands its range of applications in the biomedical area with demand for biodegradable films under physiological conditions.

Zein being the main endospermal storage protein (used in growth) of maize. This can be classified based on its solubility and molecular weight into α (19 and 22 kDa), β (17-18 kDa), γ (16 and 27 kDa) and δ (10 kDa). Of all types of zein, α-zein accounts for 75 to 85% of the composition of this protein, with γ-zein being the most abundant, approximately 20%. Zein is particularly rich in hydrophobic amino acids but deficient in polar or ionizable amino acids. Making zein slightly insoluble in pure water, but soluble in binary solutions of short-chain aliphatic alcohols and water, such as aqueous ethanol (BISHARAT et al., 2017).

### GUIDED BONE REGENERATION

The concept of GBR was defined based on tissues that regenerate when cells with this capacity populate the bone defect during healing. This way, the mechanical restriction of the soft tissue allows osteogenic cells to stimulate the formation of new bone tissue (CORTELLI et al., 2005 apud AYUB, 2011).

GBR is a surgical technique, which is based on the concept of osteopromotion with the use of a physical barrier in order to prevent the migration of undesirable cells originating

from the connective and epithelial tissues, enabling bone formation (PEREIRA et al., 2012).

Guided Bone Regeneration is mainly used in the maxillofacial region, based on the application of a barrier membrane to prevent the appearance of bone defects, not allowing non-osteogenic tissues to interfere with bone healing (TURRI et al., 2016).

For repairs of bone defects in the oral cavity, GBR uses autogenous grafted materials, which is the most used and membranes as a barrier through a surgical procedure to stimulate and guide the growth of new bone in places with defects (GEISTLICH FARMA DO BRASIL).

The ROG technique is used, for example, to restore bone in case of defects such as fenestration or tissue dehiscence around the implant, to balance larger imperfections of the jaw or to prevent bone resorption after tooth extraction in defective sockets (GEISTLICH FARMA DO BRASIL).

Usually when a periodontal infection causes some damage to the alveolar bone, surgery is resorted to; however, it only presents clinical improvements, since scars are often formed at the site, thus causing a repair with the formation of a large junctional epithelium and not bone regeneration itself (SCULEAN et al., 2015).

Even though the surgery for oral bone defects is extremely minor and leaves the least amount of damage possible, there is still the possibility that other internal factors may cause reabsorption of the alveolar ridge. On the other hand, the larger the bone defect, the larger and more complex the surgery will be, often requiring autogenous grafts, thus increasing patient morbidity (SALOMÃO; SIQUEIRA, 2010).

## BIOMATERIALS AND THEIR USE AS BARRIER MEMBRANES

Knowing that the epithelium migrates an average of 3 to 4 times faster than the connective tissue, Bjorn in 1961 proposed that if there was something that prevented the invasion of the epithelial tissue to a certain area, tissue regeneration would be possible, initiating the concept of barrier membranes to guide cells that must provide bone regeneration (COSTA et al., 2016).

The first materials approved for clinical use were non-resorbable membranes, requiring secondary surgery for their removal. They have the ability to maintain their structural integrity, dimensional stability and shape allows the surgeon complete control of its application and reduction of effect variations, its function is temporary and once completed it is removed (COSTA et al., 2016).

The first to enter the market was made of expanded polytetrafluoroethylene (e-PTFE), a porous Teflon membrane. Then, other non-absorbable membranes emerged, such as polytetrafluoroethylene, silicone (Biobrane®) and cellulose membranes (COSTA et al., 2016).

For Costa et al. (2016), there are several non-resorbable materials available on the market, such as:

a) Millipore® filter: operates as a mechanical barrier favoring root repopulation by means of cells originating from the periodontal ligament, between the gingival and epithelial connective tissue and the root surface;

b) Biobrane®: semipermeable silicone membrane, with good adhesion, mechanically bonded to a flexible nylon fabric covered with hydrophilic collagen.

The biggest disadvantage of non-resorbable membranes is the need for secondary surgical intervention for their removal (COSTA et al., 2016).

Absorbable membranes can be made of collagen (Geistlich Bio-Gide®), polylactic acid (Guidor®), polyglactin 910 (Vicryl®) and

polylactic glycolic acid (PLGA®) (COSTA et al., 2016).

The benefits of using an absorbable membrane are shown by immobilizing the clot at the tooth extraction site, stabilizing and promoting increased wound healing and its chemotactic ability to attract fibroblasts. However, if not correctly fixed, it allows movement and reabsorption, causing disruption of the clot surface, leading to the development of soft tissue between the membrane and the clot, making bone repair difficult (COSTA et al., 2016).

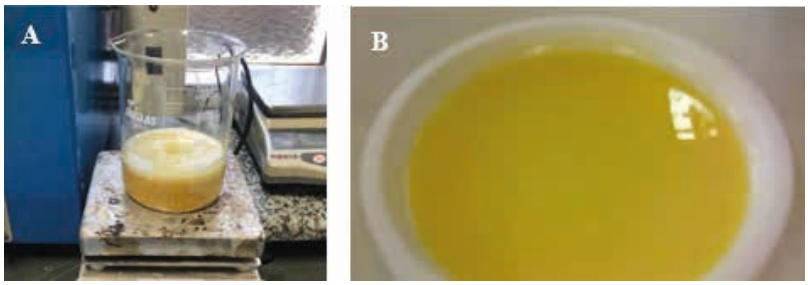


Figure x: Sample and isopropyl alcohol with 88% ratio 8: 1 liquid / solid in drink, stirring and heating at 60 to 65 ° C (A), sample after filtering, poured into the petri dish (B).

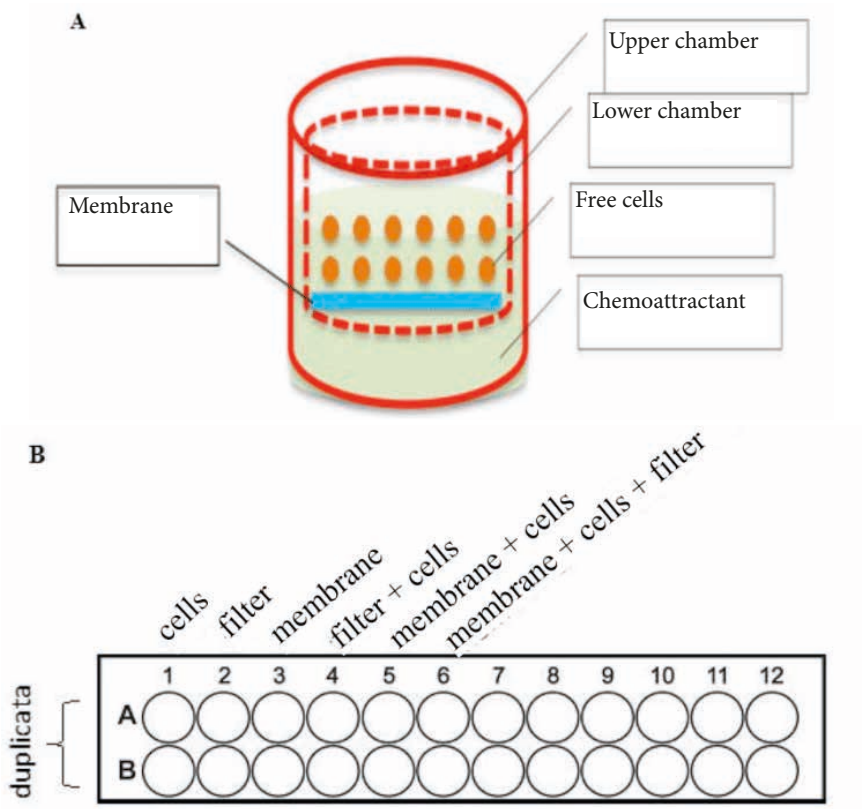


Figure X: Cell migration through the membrane. The experiment started with the insertion of the membrane in the apical compartment of the Transwell® plates, followed by the addition of the cell suspension on the membrane, in order to deposit the cells and monitor their migration (or not) (A). Scheme of the experiment in the 96-well plate, was performed with a well containing only the cells (for growth control), a well containing the filter with cells sown on its surface, a well containing the filter without cells, a well containing the membrane of zein with cells sown directly on its surface, a well containing the zein membrane placed on the filter, on which the cells were sown for analysis of their barrier action to cell migration (B).

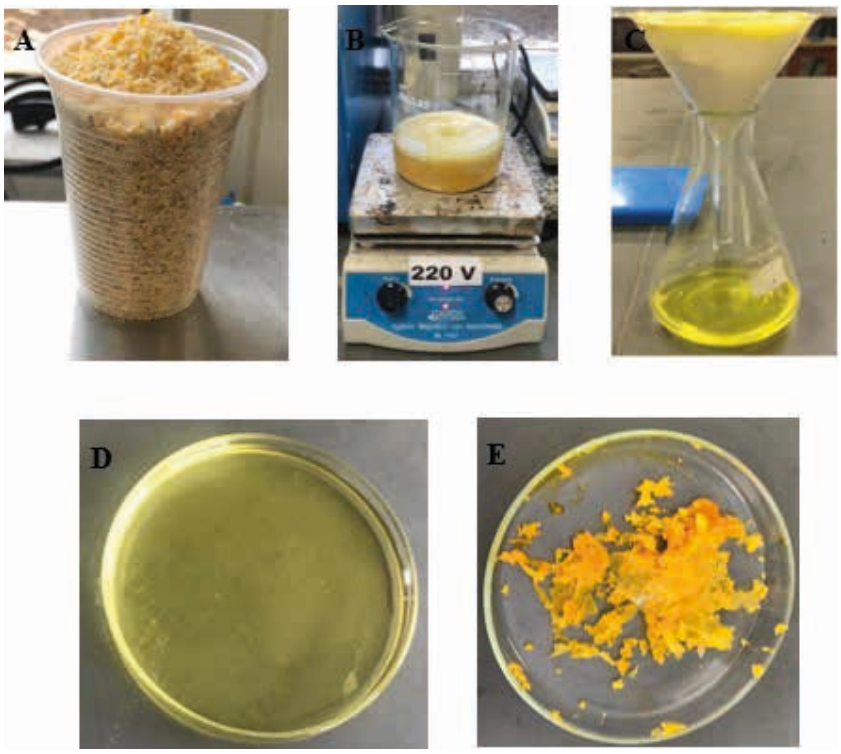


Figure x: Steps in the process of protein extraction and membrane preparation. 100 grams of the dry corn grind samples (Shimadzu scale BL3200H 3200g X 0.01g) (A), sample and 88% isopropyl alcohol were added in a beaker, heated to a temperature of 60 to 65 ° C, stirred in magnetic stirrer (Magnetic stirrer with heating - NI 1103 - Novainstruments) (B), then the contents were filtered with the aid of a beaker, funnel and filter paper (Unifil - 18.5 cm) to separate the liquid from the solid (C), the sample was then poured into a petri dish, placed to precipitate in a common refrigerator at -18ºC, for 24 hours (D), the precipitate was removed with the aid of a spatula and added absolute hexane (alkane hydrocarbon) - QHEMIS) in mechanical agitation to remove fats (E).

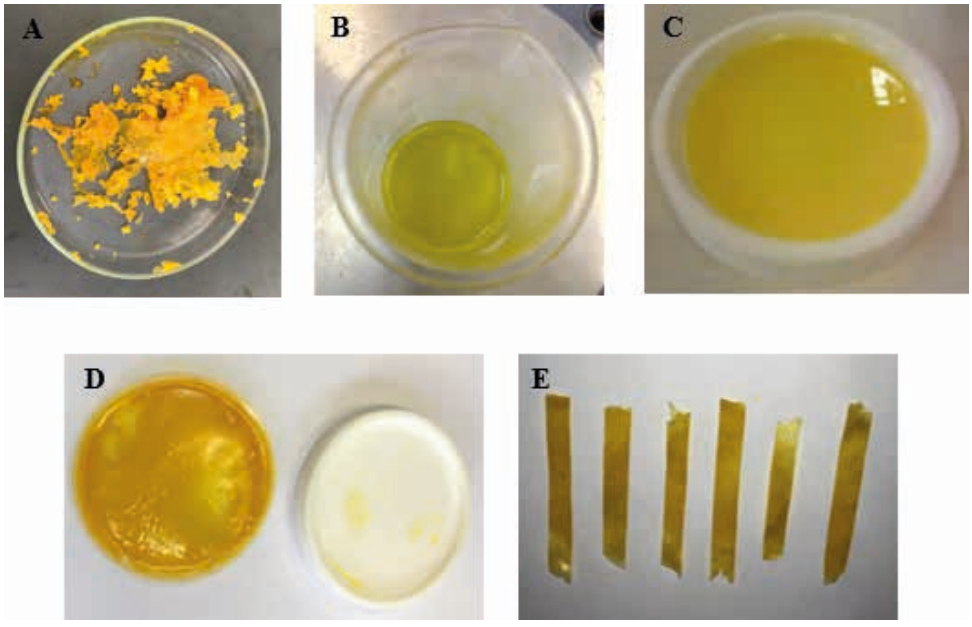


Figure x: After extracting the protein (A), it was dissolved in 70% ethanol (B) and dried in an oven at 45ºC (C and D), and then pressed at 70ºC, generating the films (E).

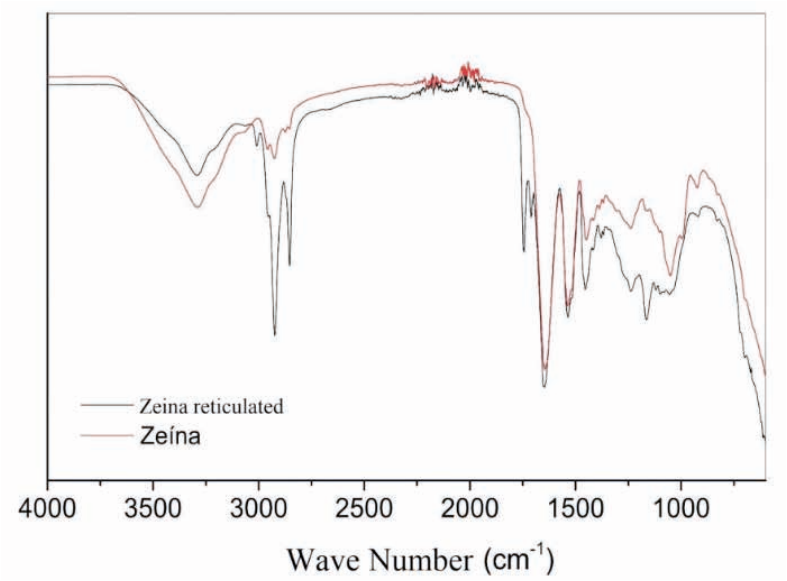


Figure 15: FTIR chart.

Figure x: The differences shown in the graph between the non-cross-linked and cross-linked protein are found in the regions 1750 to 1600 cm-1, indicating the presence of the C = O group. At 1400 cm-1, they indicate C2H4O acetaldehyde and C5H8O2 glutaraldehyde side chains. The C = O group is part of the structure of glutaraldehyde and may indicate its presence.

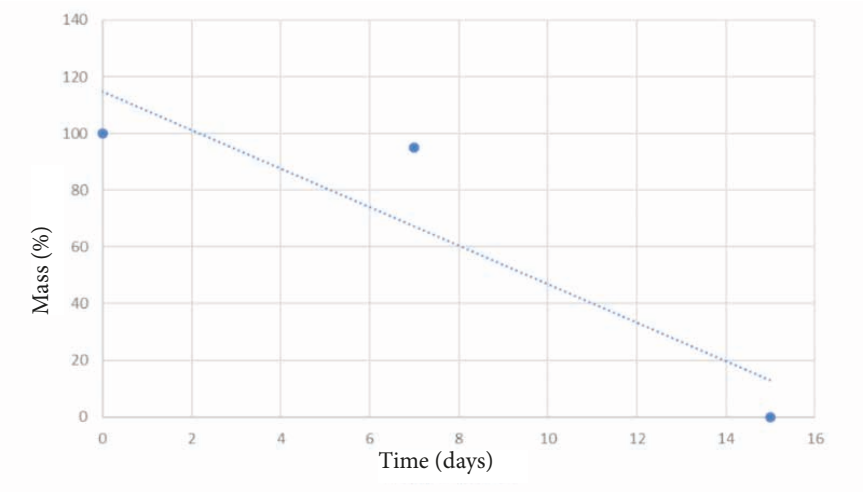


Figure x: Hydrolytic degradation of the non-crosslinked sample, showing the percentage in mass as a function of time in days.

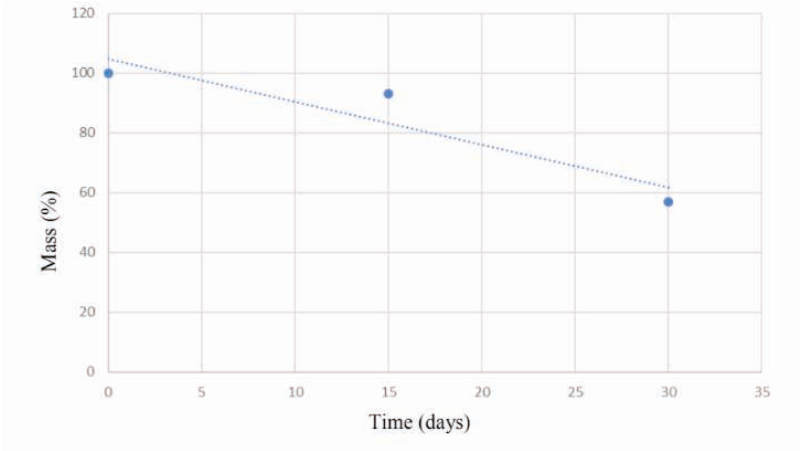


Figure x: Hydrolytic degradation of the cross-linked sample, showing the percentage in mass as a function of time in days.

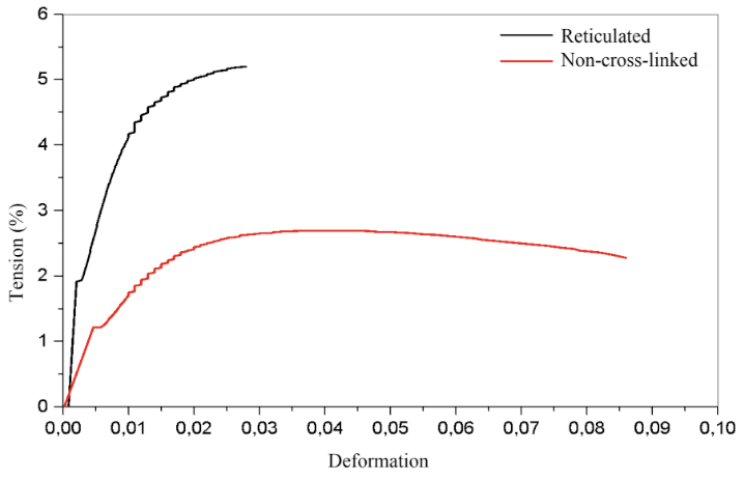


Figure x: FTIR spectra of the zein films, with the red line being the non-crosslinked sample and the black one being the crosslinked one, comparing the resistance forces on a tension between the two.

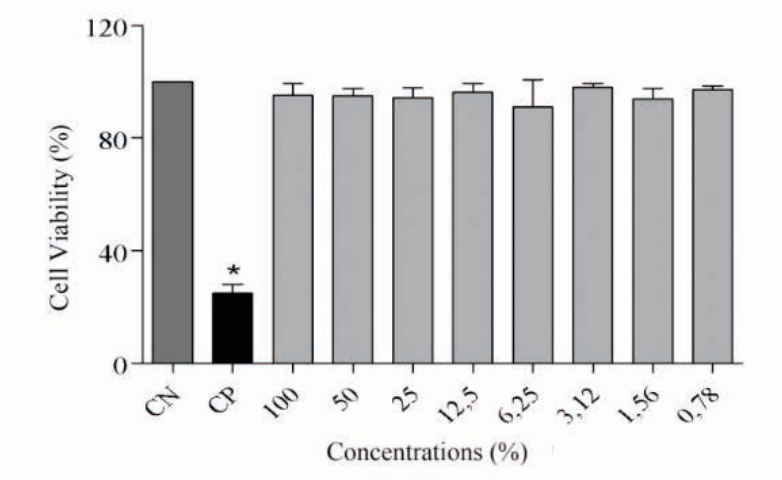


Figure x: Values are presented as mean and standard deviation. CN: negative control (DMEM culture medium, supplemented with 10% fetal bovine serum, 100% cell viability); Positive control (DMSO 20%; cell viability 25%).

* Significantly different from the negative control ($p < 0.05$).