Obtention and Characterization of Collagenous Membrane Derived from Bovine Bone Cartilage

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Introduction.

Collagen represents the most abundant animal protein. It has two important characteristics as a biomaterial: (a) low levels of allergenicity; and the capability of reorganization in the original structure, as in the native tissue with similar properties. The membranes made of collagen can be used to recover bone grafting materials in guided tissue regeneration procedures avoiding that soft tissues, as muscle and mucous, invades the site where the osseointegration occurs. The collagen derived from bovine bone cartilage shows great viability to generate protective membranes to be applied in bone tissue regeneration procedures.

Materials and Methods.

The developed collagen membranes are derived from bovine cartilage by separation and cleaning by chemical procedures. The process to obtain such membranes has four fundamental steps: (a) dissolution of the bone cartilage collagen in acetic acid solution; (b) neutralization of pH with ammonium hydroxide solution; (c) drying at 25°C in the desired mould; and (d) γ Ray irradiation. The membranes were prepared in various formats and dimensions. For characterization was realized the tests:

Mechanical Assay: Were prepared strips of membrane with dimensions 5mmx30mmx0,1mm. The test was realized until rupture in two conditions with the membrane dried and the membrane hydrated in its middle part. For the realization of the test was utilized at Instron 4400R machine, load cell of 50kg and velocity of the tensile test equal to 50 mm/s.

Hygroscopicity Assay: The amount of deionized water absorbed by the membrane structure was measured in determined periods. Before immersion procedure, all the samples were dried and weight. After the immersion periods, the excess of water was removed from the samples by a filter paper and then were obtained their wet weights.The absorbed water was calculated by the equation 1:

$$\%water = \frac{(m_w - m_d)}{m_w} \times 100 \qquad (1)$$

m_w and m_d are the wet and dried weights, respectively. The obtained results are representative of four independent measurements.

Cytotoxicity Assay: Carried out by neutral red uptake methodology, utilizing samples extracts dilutions in contact with NCTC L929 cell culture in 96 wells microplates. The medium culture was MEM supplemented with 5% Calf bovine serum. Extract: immersion of samples in MEM for 24h at 37°C.

Controls: positive (0,02% phenol solution) and negative (no toxic PVC pellets). The cell viability was measured by incorporation of neutral red by viable cells and in the final procedure cell rupture and DO measure on 540nm filter of spectrophotometer Sunrise – Tecan ELISA reader.

Results and Discussion.

Different dimensions and formats can be produced with collagenous membrane derived from bovine bone cartilage as shown in Figure 1.

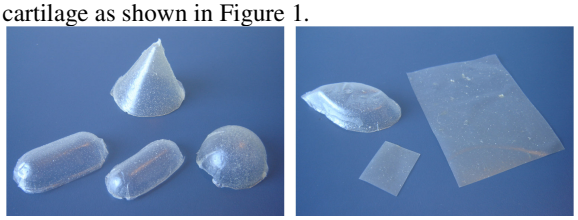

Fig. 1 - Collagen membranes in some formats.

The strips of collagen structure were elongated by a uniaxial load until the rupture charge. The table I shows the resulting effective stresses.

Table I - Effective Rupture Stress.

EFFECTIVE STRESS (kg/mm2)	
DRIED	**WET**
2,58±0,65	0,39±0,10

The water absorption capacity of the membranes can be seen in the Figure 2, where it is observed that in 10 minutes occurs a great absorption of water and after 50 minutes of immersion there´s a region of stability.

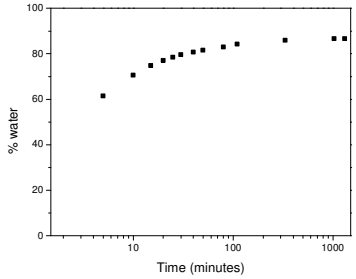

Fig. 2 - Water absorption by immersion period of the membranes.

The collagenous membrane shows viability curve above IC50% line and are considered no cytotoxic (Fig. 3).

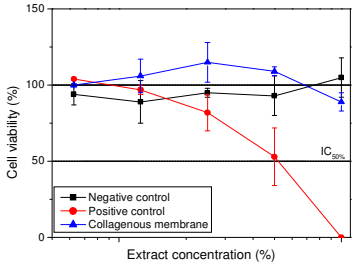

Fig. 3 - Viability curve of Collagen Membrane in the in vitro cytotocicity assay by the neutral red uptake method.

Conclusions: The most important advantage of the obtained with the chemical process described is the capacity of the membranes to produce in any dimensions and shapes, with good dry and wet mechanical resistance and stable structure, characteristics that can indicate them to be used in all surgical cases in correction of bone defects.

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