PRECLINIC TEST OF COLLAGEN MEMBRANES

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ABSTRACT

Biocompatibility tests of materials proposed for medical applications are essential for determining its ability to be accepted in the organism which will be implanted. At the present time, the studies needed between others are in vitro (cell) and in vivo: preclinical (animal) and clinical (human) tests.

In this work were used porous membranes of collagen (type I) from inorganic bovine bone Nukbone®, to evaluate their biocompatibility in preclinical phase, using as animal model: rabbits.

The collagen porous membranes were tested in the back of rabbits, to which were withdrew a circle of skin where were implanted the membranes. It was found that the repair tissue process was faster and better than in other case, furthermore the new skin showed a better quality. Collagen porous membranes from Nukbone® were biocompatible.

INTRODUCTION

Collagen is a multifunctional family of proteins of unique structural characteristics and probably the most abundant animal protein in nature. It is estimated that collagen accounts for about 30 % of the total human body protein[1]. Collagen contributes to mechanical properties and tissue integrity[2]. Collagen is located in the extracellular matrix of connective tissues.

Currently known 25 different types of collagen, which differ only by their number and sequence of amino acids, collagen type I being the most abundant, as is found in bone, skin, tendon, among others and is the same for all mammals . This feature is one that interests us take to find a biocompatible material, readily available, high purity, good mechanical properties, adaptable to situations surgical and medical required to solve problems such as loss of skin burns or trauma, used as mesh in case of hernias, like separation between organs and tissues, increased volume in connective tissue, a guide for regeneration of cartilage, or like haemostatic[3-6] sponges among others.

The collagen may come from different sources and from different organs and tissues (bone, tendon, skin, etc.) from fish (i.e. salmon, shark) and from mammals, in particular from domestic mammals like bovine and pigs[7-9].

This paper aims to assess the biofuncionality of the collagen porous membranes obtained from the bone matrix of bovine bone named Nukbone®[10], in rabbits with skin lesions at different times of evolution.

The collagen membrane was used as tissue support to reduce the time repair of damaged and avoid dehydration and infection of the tissue, which was observed at different times of evolution in experimental animal model (preclinical study phase).

Up to the present there are different presentations of collagen that can be purchased commercially like: Fibrogen, Bioderm, Tegaderm, etc. [11-13].

The protocol was used anesthetic ketamine 25 mg/Kg IM, xylazine 5 mg/Kg IM Pentobarbital sodium and 20 mg/Kg IV[17]. After the surgical procedure was used analgesic Dipyrone Vet 500 mg/kg every 24 h/2 days IM (trade name and laboratory in all cases).

Before his placement, the collagen membrane was hydrated with saline solution to 0.9% in order to obtain greater flexibility, see Figure 2 and then was placed on the injury, because their properties haemostatic, it was not necessary to remove or clean hematopoietic tissue from the wound, see Figure 3. To contain the membrane in its place, was necessary sutured it in four sites using Polypropylene (Prolene, Ethicon) 5/0 as shown in Figure 4.

The times of assessment for the membrane were 7, 14, 30 and 45 days after surgery. For the seizure of regenerated tissue, the animals were sacrificed with an overdose of pentobarbital (60 mg kg).

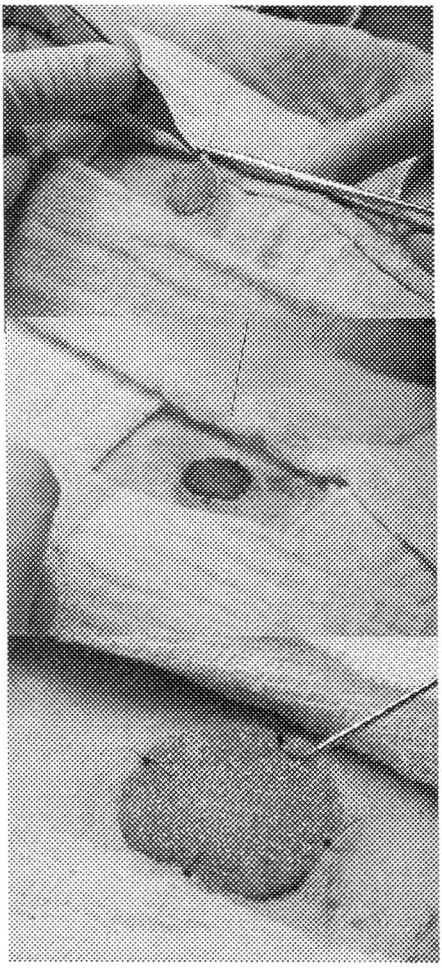

Figure 2a). The membrane has the ability to adapt to the wound.

Figure 2b). It is possible observe the haemo static ability of the collagen membrane.

Figure 2c). Sutured membrane can be seen over the wound.

RESULTS

The animal model used for evaluating the biofuncionality of collagen membrane was acceptable, the implantation did not move in any case.

The treatment, the surgical procedure and anesthetic used, were easily applicable in this specie. There was no type of infection.

It was not necessary use dressings or meshes to cover the membrane, because the biomaterial was designed according to the skin lesion leaving the membrane attached perfectly with the skin of the animal, not allowing the subcutaneous tissue get in contact with the outside world, that could leave it expose to an invasion of microorganisms and to a possible infection.

One advantage of using the dorsal skin of the animals to place the membranes is facilitating the clinical observations without any treatment to the animal that could move the membrane.

Before surgery, in all cases, the membrane was hydrated with sterilized saline solution in order to keep manageable, because when the membrane is not wet, it is very rigid. Once hydrated the collagen membrane, this kept their mechanical properties (resistance), there was not ruptured of the membrane at the time of placing the suture between the membrane and the skin of the animal. This assured the permanence of the membrane on the site of implantation.

There were not infection, nor chronic inflammation, nor detachment or rupture of the sutured membranes in any case, during the evaluation time.

The membrane permeability was observed until 72 hours after implantation, during this time the membrane remained impermeable due to growth of epithelial tissue of the injury, which was promoted by the use of membrane that functioned as scaffolding.

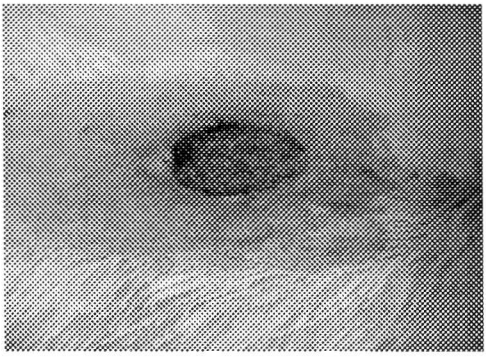

Figure 3. It can see the membrane implanted after 72 h after surgery.

There was not a contraction of the wound in any case, which would be a normal phenomenon especially for circular injuries that takes 30% more time to heal than any other geometric shape of injury.

During times of evaluation of the membranes, these remained in their place; these biomaterials retained their architecture but gradually became dehydrated.

CONCLUSIONS

The type I collagen is a protein common in all mammals, their compatibility has been proven in many published works.

This study showed that the collagen sponge obtained from the bone matrix from bovine bone presents very good functionality to establish as a substitute for skin, because the animal remained healthy and assisted in the regeneration of the skin over it. The animal model proposed for evaluating the collagen membrane was appropriate and easy to handle.

The biomaterial proposed in this work has the potential to be used in different medical procedures requiring the use of scaffolding or support cell (guided tissue regeneration) as well as transient skin covered in burns, skin ulcers or in specialties such as dentistry, plastic and reconstructive surgery, orthopedics, urology, vascular surgery, tissue engineering, etc.

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