

Use of Nanoskin for volume replacement of the eye socket

Nanoskin: uso para reposição de volume na cavidade anoftálmica

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ABSTRACT

Objective: The aim of this study was to evaluate the biocompatibility of Nanoskin for replacing volume in enucleated or eviscerated anophthalmic sockets of rabbits. **Methods:** An experimental study was carried out using enucleated or eviscerated rabbits, which received Nanoskin implants (Innovatecs®, São Carlos, Brazil), a cellulose produced by a bacteria (*Acetobacter xylinum*) using green tea as substrate. Implants of 10mm diameter/5mm of thickness were used placed in enucleated (G1) or eviscerated (G2) anophthalmic sockets of 21 rabbits. They were clinically examined daily, sacrificed at 7, 30 and 90 days after surgery and the material was removed and prepared for histological examination. **Results:** There were discrete signs of inflammation in the immediate postoperative period, with no evidence of infection or extrusion in any animal. However apparent reduction of volume during the trial period occurred. Histologically both groups were similar, with inflammatory cells (mainly monocytes and neutrophils), fibrin and hemaceas at 7 days postoperatively. The Nanoskin was presented as small pink spheres, with small gaps between them and permeated by few inflammatory cells. These cells have changed over the study, at 30 days multinucleated giant cells and mature fibroblasts that permeate the implant were observed. At 90 days, the structure of the implant was disorganized, amorphous, with necrotic debris and ovoid areas covered with thin pink membrane that seemed to cluster, empty or filled with no cellular pink or gray material. **Conclusion:** Nanoskin caused an inflammatory reaction leading to reabsorption and reduction of implant volume. New formulations should be studied in order to have a permanent product to repair the anophthalmic socket.

Keywords: Anophthalmos/therapy; Biocompatible materials; Cellulose/metabolism; Prostheses and implants; Eye enucleation; Eye evisceration; Rabbits

RESUMO

Objetivo: Avaliar a biocompatibilidade da Nanoskin para reposição de volume em cavidades enucleadas ou evisceradas de coelhos. **Métodos:** Estudo experimental, utilizando implantes de Nanoskin (Innovatecs®, São Carlos, Brasil), celulose bacteriana produzida pela bactéria *Acetobacter xylinum* tendo como substrato o chá-verde. Implantes de 10mm de diâmetro/5mm de espessura foram colocados em cavidades enucleadas (G1) ou evisceradas (G2) de 21 coelhos, avaliados clinicamente todos os dias, sacrificados aos 7, 30 e 90 dias após a cirurgia. O material foi removido e preparado para exame de microscopia óptica. **Resultados:** Sinais flogísticos discretos no pós-operatório imediato, não tendo sido evidenciados sinais infecciosos ou extrusão de nenhum implante. Houve aparente redução do volume ao longo do período experimental. Histologicamente ambos os grupos foram muito semelhantes, apresentando aos 7 dias células inflamatórias (predominantemente monócitos e neutrófilos), rede de fibrina e hemácias. A Nanoskin apresentava-se como pequenas esferas, de cor rósea, com pequenos espaços entre elas, permeados por escassas células inflamatórias. As células inflamatórias se modificaram ao longo de período experimental, sendo possível observar aos 30 dias células gigantes multinucleadas e fibroblastos maduros permeando o implante. Aos 90 dias, a estrutura do implante apresentava-se desorganizada, amorfa, com restos necróticos e com áreas ovoides, revestidas por fina membrana rósea, que pareciam se agrupar, vazias ou preenchidas por material acelular, róseo ou acinzentado. **Conclusão:** A Nanoskin provocou reação inflamatória que levou à reabsorção e redução do volume do implante. Novas formulações devem ser estudadas a fim de ter um produto que seja permanente para reparo da cavidade anoftálmica.

Descritores: Anoftalmia/terapia; Materiais biocompatíveis; Celulose/metabolismo; Próteses e implantes; Enucleação ocular; Evisceração do olho; Coelhos

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INTRODUCTION

Since the development of integrated implants in the 1980's last century the scenario of the anophthalmic eye socket reconstructions is changing, and today there is room for evaluation of proposals of biomaterials for use in anophthalmic eye socket.

Nanoskin is a bacterial cellulose formed by nanofibrils synthesized from the bacterial proliferation, with energy consumption, which can arise from the polymerization of glucose.¹

Hemicellulose film Nanoskin® was developed by Innovatecs® by means of a biotechnological process. It comprises a mixture of bacteria growing on various substrates such as yeast-cane broth or green tea, providing a culture medium in which the biological organism *Acetobacter xylinum*, a Gram-negative bacterium, develops the product with biodegradable, biocompatible, non-toxic and non-allergenic characteristics. Its micro-porosity reduces water loss and retains adequate moisture, allowing oxygenation of the injured area, and creating an ideal granulation and epithelialization environment (available at www.bionanofuture.com).

It is a byproduct where bacteria use sucrose for producing a pulp-like material, or the bacteria have properties to produce a bio-polysaccharide resulting in thin films and other compositions which can be used for various purposes.²

Despite the good tissue response obtained with several different materials, there is still no Brazilian biomaterial that has reached the market for volume replacement in anophthalmic eye socket, and Nanoskin could be a proposal in this sense.

Thus, the present study was carried out in order to assess the biocompatibility of Nanoskin spheres deployed in eviscerated or enucleated eye sockets of rabbits.

METHODS

Experimental and randomised study in which 21 rabbits of the species *Oryctolagus cuniculus*, of both genders, aged between 3 and 6 months were used. The animals were given by Biotério Central da Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), located on Campus Botucatu (SP). The study protocol was approved for implementation by the Ethics Committee of the institution. Sterilization of the material was with Gamma Ray - 7,3KGY, according to the International Standart ISO 11737-2:2009(E) and U.S PHARMACOPEIA/ NATIONAL FORMULARY-USP 34/NF 29,2011.

Groups and experimental moments: the rabbits were divided at random into two groups composed of 21 animals who were anesthetized and then had their eyes eviscerated (Group 1) or enucleated (Group 2), with replacement of the volume lost using implants comprising Nanoskin (Innovatecs®, São Carlos, Brazil) of 10 mm diameter / 5 mm thickness sterilized by the manufacturer and provided at no cost to the experiment. After the surgical procedure, once again there was randomization for the decision to compose experimental moments: moment 1 (M1) when 7 animals from G1 and G2 remained 7 days with the implants in the anophthalmic eye socket; moment 2 (M2) containing 7 animals of G1 and G2 who remained 30 days with the implants in the anophthalmic eye socket, and moment 3 (M3) when 7 animals in G1 and G2 remained 90 days with the implants in the anophthalmic eye socket.

All surgical procedures were performed in at Laboratório de Cirurgia Experimental da Faculdade de Medicina de Botucatu - UNESP under antisepsis and asepsis conditions, as described below: general anesthesia using Tiletamine associated with Zolazepam (Zoletil® 50, Virbac do Brasil Indústria e Comércio Ltda, São Paulo, SP, Brazil) at a dose of 50 mg/kg injected into the auricular vein; lay the rabbit on its left side, with the right eye facing up; antisepsis and asepsis of the right eye with Polyvinylpyrrolidone-iodine (Prosigma - prescription pharmacy Prosigma Ltda ME, Cambui, MG, Brazil), placing ophthalmic and blepharostat field; peribulbar and subconjunctival application of 1.0 ml of lidocaine hydrochloride with epinephrine (xylocaine with epinephrine 1:200,000 AstraZeneca Inc. - Mississauga - Canada) to complement the analgesia; corneal-scleral opening with 15 scalpel blade; evisceration (G1) or enucleation (G2) of the content of the right eye and placement of Nanoskin implants; closing the incision with continuous stitches with 6-0 nonabsorbable braided (Mersilene, Ethicon Incorporation - Johnson & Johnson do Brasil Indústria e Comércio de produtos para saúde Ltda, São Paulo, SP); instillation of 0.1 ml of Ciprofloxacin Ophthalmic Solution 3% (Alcon Laboratories, SP, Brazil).

The animals were kept in individual cages, under controlled conditions of temperature, humidity and lighting, and received water and feed *ad libitum*, being killed 7, 30 and 90 days after the initial surgery with an overdose of Zolazepam (Zoletil® 50, Virbac do Brasil Indústria e Comércio Ltda, São Paulo, Brazil) intravenously.

After death, the implants with surrounding tissue were removed, and then the material was prepared for histological examination with paraffin blockage, and staining with hematoxylin-eosin following the routine laboratory protocol.

The biocompatibility of the implants was studied through daily clinical examination, taking as parameters the local exam of the orbital cavity and data for the overall health of the animal, as measured by the attitude in the cage, the appetite and general activity, and by histological exam, assessing the inflammatory reaction around and inside the biomaterial.

RESULTS

Clinical assessment: during the clinical follow-up, all the animals remain healthy, being normally fed. Two animals died during the intraoperative period due to the anesthetic effect. For the three animals with behavior indicating pain, it was necessary to administer analgesics in the postoperative period during the first 3 days.

All rabbits showed signs of inflammation in the orbital cavity, especially in the first days after surgery, with the presence of acolade yellowish secretion in the region affected, but none of them developed infectious signs, suture dehiscence or extrusion of the implant.

During the animal observation period there was no apparent reduction in the volume of the orbital cavity, showing reduction of the implant volume.

Histology assessment (Figure 1)

G1M1: identification of scleral cover, inflammatory cells (usually neutrophils), red blood cells and a loose network of fibrin between the sclera and the implant. The implant material was identified as refractive, consisting of pinkish circular, round or elongated formations disposed in the central area of the sclera.

Inflammatory cells were present in all rabbits assessed, and many necrotic debris around the implant.

G1M2: exuberance of inflammatory cells, especially polymorphonuclear and mononuclear cells, macrophages and multinucleated giant cells forming granulomas. The new vessels contain red blood cells. Fibroblasts are organized within the sclera housing Nanoskin. Empty ovoid or circular areas within the sclera. Presence of necrotic cells and calcification in all slides analyzed.

G1M3: the empty areas increased in size probably influenced by other smaller ones. Nanoskin showed as rounded and refractile granules in some animals, and was absent on others (possibly due to loss of material during extraction or by absorption thereof). Pink or gray amorphous material was present inside the empty cavities. Giant cells, macrophages and granulomatous reaction involving parts of Nanoskin. Presence of necrotic cells and calcification in all slides analyzed. There was no formation of fibrosis.

G2M1: it was difficult to identify the Nanoskin implant in enucleated cavities. The inflammatory reaction that occurred in the eviscerated animals was similar to that happened in the enucleated ones, including the most exuberant reaction concentrated around the Nanoskin.

G2M2: all the characteristics observed, including formation of granulomas containing giant cells in the regions where Nanoskin is found, occurred in a similar way to what occurred in G1M2.

G2M3: exuberant inflammatory reaction around the implant which was not evidenced in all animals, as well as in G1M3.

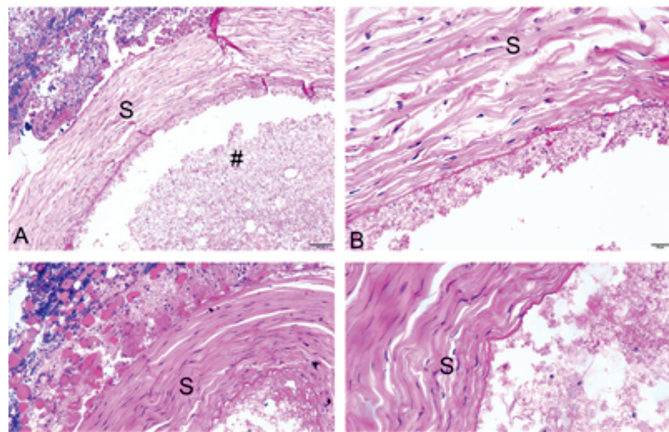


Figure 1: A, B- G1M1: loose net of fibrin (#) between parts of the implant, identified as rosy material, disposed in the central area of the sclera (S). Inflammatory cells of acute phase permeating the implant; C, D - G1M2: inflammatory cells in contact with Nanoskin. Circular or ovoid, pink or gray areas inside the sclera (S)(*); E,F: G1M3: large empty areas inside the empty cavities (@). Giant cells, macrophages and granulomatous reaction (g) involving parts of Nanoskin.

DISCUSSION

A long technological challenge has been traversed until the production method of Nanoskin, material resulting from the synthesis process and which, thus, is not limited, as it would be in the case of other materials such as natural hydroxyapatite.³

The strong initial appeal of integrated implants³ was the starting point so that new materials were studied⁴⁻⁶, opening a wide range of research seeking for the ideal biomaterial.

The present study showed that Nanoskin is a biomaterial that can be well tolerated by the orbital cavity, not having caused any death or showing that the overall health of the animal had been affected, despite the need for biochemical and histology exams of target organs to confirm that.

Another important point was the non-observation of clinical signs showing that Nanoskin was intolerable to the orbital tissues. The observation periods of 7, 30 and 90 days allowed the observation of early and late healing factors to the clinical exam. There should have been development to dehiscence of the conjunctiva and/or sclera, closely associated to technical problems in the surgery⁷ and which did not occur. And also, there were no later extrusion of the implants, both in G1 and G2.

The histological exam showed little inflammatory reaction in early stages in the eviscerated and also enucleated cavities, with the appearance of giant cells and phagocytosis processes in later times. This fact, along with the observation of pinky areas supposedly formed from the liquefaction of biomaterials, lead to suspicion of absorption of the material implanted. This process will invariably lead to a gradual reduction of the implant volume, unwanted phenomenon when working with volume replacement in the orbital cavity⁸ and already noted with some types of synthetic hydroxyapatite.^{4,9}

In addition to the biological phenomena arising from the implementation of the biomaterials proposed for use in the anophthalmic eye socket, we must consider the stability and ease of the methods in the production process, the availability of materials to be used in the manufacture and the end price of the product. Chitosan spheres implanted in eviscerated cavities of rabbit cause tissue reaction based on regenerative phenomena and with little inflammation, besides not systemically affecting the animals.¹⁰ However, these implants are difficult to manufacture, which puts chitosan as a unless proposal.¹⁰

Nanoskin implants have simple and inexpensive production. However, the current formulation is not advised to replace the volume in the eye socket, as there was reabsorption of the material and reduction of the volume implanted. New tests will be performed in order to modify the formulation of Nanoskin spheres in order to make it a non-absorbable material. It can only be used when stability is safely maintained, without the formation of granulomas containing giant cells.

CONCLUSION

Nanoskin is a biomaterial that may be applied to volume repair in the anophthalmic eye socket. However, changes in the manufacturing process are required in order to maintain the stability of the product. New researches should be provided in this regard.

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