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Review

Chitosan – a promising biomaterial in veterinary medicine

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Abstract

Biomaterials originate from natural substances and are widely used in medicine. Although they have to satisfy many conditions to be useful for treatment, more and more research is carried out with new types of biomaterials that can help replace various tissues such as tendons and bones. Chitosan is a very promising material, revealing unique features, which makes it useful for veterinary medicine – antimicrobial activity, biocompatibility, biodegradability. It is also known as good scaffold material, especially when combined with other polymers. This article describes chitosan as a biomaterial and tissue engineering scaffold with possible applications in veterinary medicine.

Key words: chitosan, scaffolds, biomaterials, tissue engineering

Biomaterials – definition

Biomaterials are defined as active substances capable of interaction with the surrounding tissues, without causing an immune response (Shigemasa et al. 1996). Research on the development of bioengineering (the branch of science dealing with the design of biomaterials) has been developing for decades and are still in progress – there is no perfect material yet, mainly because of the multitude of conditions that must be complied with by such a material, in order to be used in the medical field.

Biomaterials – characteristics

An ideal biomaterial has to be biocompatible with the surrounding tissue and cause no allergenic response. Other important aspects are its antimicrobial activity, the ability to inhibit inflammation and stimulate healing; it should also relieve pain. There are also features which are important from the technical point of view, especially for the field of veterinary practice – the ability to be used in non-hospital conditions, ease of application and low price increases the possibility of its application. These features are designed to reduce the time of treatment, the frequency and the amount of drugs used (bearing in mind their side effects) (Shigemasa et al. 1996). Moreover, they create new possibilities of application and multiply treatment options, better suited to particular cases.

During researches, it was quickly noticed that the materials derived from natural compounds are better accepted by the organism than those synthesized synthetically (Shigemasa et al. 1996), because they are built from the same material as tissues and cells,

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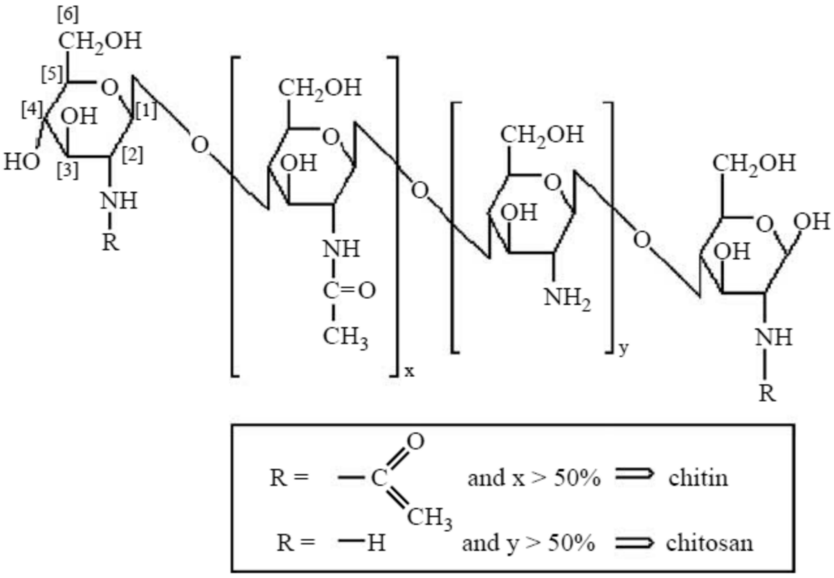

Fig. 1. Schematic representation of chitin and chitosan depicting the co-polymer character of biopolymers (Khor 2001).

so that the organism does not reject it as foreign (biocompatibility). A new branch of science – bioengineering – soon developed, focusing on biomaterials of natural origin.

Objective of the manuscript

This article focuses on one biomaterial – chitosan.

Use of biomaterials and their types

Biomaterials are used in many areas of medicine – from dressings through complementing tissue defects, supporting tissue regeneration and transplantation. In veterinary medicine, however, and in particular the equine medicine, high hopes are raised in the field of orthopedics – especially in the treatment of tendons and joints. This branch is still looking for new techniques for the recovery, regeneration and rebuilding of damaged tissues. When analyzing the biomechanics of joints and tendons, not only the characteristics required of the biomaterial must be taken into account, but also the main features of the tissue: strength and flexibility.

Currently, biomaterials that have been proposed and tested on lab animals are: polycaprolactone (Reed et al. 2009), polyethylene glycol (Briggs et al. 2009), polyvinylalcohols (Lee et al. 2009), polyurethane (Shin et al. 2009), collagen (Lin et al. 2008), silk (Silva et al. 2008), alginate (Park et al. 2009, Penolazzi et al. 2010), starch (Martins et al. 2009) . The most interesting, however, is chitin, a substance of animal origin which is part of the external shells of invertebrates and the cell walls of bacteria and fungi (Tuzlakoglu et al. 2004, Majima et al. 2007). Its features are closest to tendon properties.

Characteristics of chitosan

Chitin is a polysaccharide composed of N-acetyl-D-glucosamine subunits and was described in 1811 by Braconnot (1811). However, in 1859, Rouget (1859) described a derivative of chitin – chitosan (Fig. 1), which can be found in the cell walls of fungi of the genus *Mucor ruoxii*. Afterwards, he obtained chitosan by deacetylation of chitin. Both materials have proven useful and promising in medical applications. However, the following 150 years of research indicated that chitosan not only meets the conditions for good biomaterial, but also has features that are better than in other biomaterials – a unique antibacterial activity, non-allergic response, oxygen permeability (breathability), biodegradation to relatively neutral oligomers by lysozyme (Cunha-Reisi et al. 2007), (an enzyme found naturally in the cells of the body) (Shigemasa et al. 1996) and a unique ability to accelerate healing (Muzarelli et al. 2007). Chitosan is a very flexible material – its properties can be freely modulated, depending on the degree of deacetylation (at 65% deacetylation it is known as chitosan) (Arca et al. 2008). Additionally, it has so much space available in the polycarbonate chains that ions or even entire functional groups can be attached to obtain the specific characteristics of the product (Senel et al. 2004). Moreover, it can be produced in different

Table 1. Application of chitosan of different conformation with observed results.

Chitosan conformation	Type of animals	Application	Effect	Observations	References
Wool	large and small animals	topical	Wound healing	Granulation, disappearance of pus, reduced time of treatment	(Minami et al. 1999)
Wool	dog	implantation	Immunostymulator	Induced activity of polymorphonuclear cells	(Kosaka et al. 1996)
Sponge	rabbit	topical	Bone healing	Healing induction	(Muzzarelli et al.1993)
Sponge	dog, cat	topical	Absess, bite wound healing	Healing induction	(Minami et al. 1993)
Hydrophilus sponge	sheep	topical	Bone healing	Regeneration induction	(Muzzarelli et al. 1998)
Granules	dog	topical	Wound healing	Healing induction	(Okamoto et al. 1995)
Powder	dog	topical	Wound healing	Healing induction	(Khanal et al. 2000)
Solution	rat	intra-articular to knee	Regeneration of cartilage	Chondrocyte proliferation	(Lu et al. 1999)

conformations: cotton, sponges, membranes, gels, fibers or scaffolds (Shigemasa et al. 1996, Madihally et al. 1999, Cunha-Reisi et al. 2007, Pillai et al. 2009). Over the last 40 years researches have been done on the use of chitosan in medicine, both in vitro and in vivo. Most of these researches described its use to treat wounds. This is due to chitosan's ability to activate wound granulation and reorganization of the wound – it stimulates inflammatory cells: leukocyte phagocytosis and the production of leukotriene B_4, macrophage phagocytosis and the production of IL-4, IL-1, TGF-β1 and fibroblast migration (Ueno et al. 2001).

Medical application of chitosan

So far, chitosan has been widely used in medicine, but fields in which it may be useful are still being discovered. In everyday veterinary medicine it is only the beginning. The applications are included in table one with reference to studies in which it has been confirmed (Table 1). Up to now, there are several fields in which chitosan is currently used: primary (hydrogels, semipermeable films, sponges) and secondary wound dressing (gauze, non-wovens) (Muzzarelli et al. 2007) and haemostatic topical agents (powders, bandages). There is also research testing this material for implants: for nerve regeneration, orthopedic applications, hernia mesh (for commercial products see below) (Shigemasa et al. 1996, Kucharska et al. 2010).

Commercial products registered

Products containing chitosan are already registered for use in humans and animals and are on the market. The largest production is in Japan (ChitiPack® S, Eisai Co., Beschitin® Unitika), USA (Tegasorb® and 3M Tegaderm®) and some European countries. In Poland there is Chitopan®, gel for use directly on the wound or Chito-derm®, ointment for skin care, both from Vet-Agro, Lublin, Poland.

Definition of tissue engineering

The most interesting and promising application of chitosan is tissue engineering. It has four targets:

1) application of biomaterial as a scaffold for cells and as an environment for their development (Choi et al. 1999).

2) cell use for artificial tissue production with or without the addition of polymers (Yamato et al. 2004, Yang et al. 2005).

3) synthesis of scaffolds with biosignals for cell development, which are normally present in the cell (growth promoters, cell differentiation and proliferation) (Fedakar-Senyucel et al. 2008).

4) combination of scaffold-based tissue made of cells and biosignals (Arca et al. 2008).

The foundation of such engineering are scaffolds. Currently, intensive research is being done on biomaterials, because they may fulfill the scaffold role (Madihally et al. 1999, Khor et al. 2003). They are not intended to replace tissue in an organism, but contribute to the normal proliferation of cells in this region, which is particularly important in tissues in which the corresponding cell arrangement determines its usefulness (tendon, bone) (Tuzlakoglu et al. 2004, Majima et al. 2007).

Biomaterial as scaffold

The biomaterial must meet a number of conditions including:

– preservation of anatomical shape and volume of the tissue,

– biocompatibility

– lack of allergic reaction,

– the breakdown products that produce no or few side effects,

– half-life time long enough for particular tissue growth,

– being mechanically strong,

– having a place for cell attachment (called pores) (Arca et al. 2008).

Chitosan, due to its characteristics, is considered a very good material for the production of scaffolds (Madihally et al. 1999, Suh et al. 2000), either alone or in combination with other polymers (eg. hyaluronate (Madihally et al. 1999). Favored for its unique properties – it stimulates fibroblast activation (Okamoto et al. 2002) by potentiating growth factors such as platelet-derived growth factor (Inui et al. 1995) or through forming polyelectrolyte complexes with serum components such as heparin (Mori et al. 1997). Also, it enhances the production of cytokines and cell migration, and stimulates the synthesis of collagen type IV (Mori et al. 1997, Majima et al. 2007, Muzzarelli et al. 2007). Additionally, it forms an oxygen-permeable biofilm on the surface of the wound, which separates it from the adverse effects of the environment. Chitosan is degraded by lysozyme – after playing its role it is reduced to oligomers.

Chitosan as a biomaterial is now being tested in many medical areas, including the induction of bone healing, cartilage defect regeneration and periodontal tissue regeneration (Arca et al. 2008).

Possible limitations

Possible limitations of the use of chitosan should be also taken into account. This material has low solubility (Chen et al. 2005). There is also continuing research on improving the strength of the scaffolds. In some publications its lack of long-term stability has been noted (Zhao et al. 2009), which in certain tissues may be an advantage, but in those which require longer development (for example tendons and bones) it is a drawback. For these reasons, chitosan is synthesized with other materials to achieve the desired characteristics of the final product – inorganic substances such as hydroxyapatite (Nie et al. 2007, Malafaya et al. 2009) or calcium phosphate (Martins et al. 2008) and other biomaterials such as gelatin (Jiankang et al. 2009), alginate (Morgan et al. 2009) and collagen (Rafat et al. 2008).

Summary

Chitosan is not a new discovery, but is now taking the attention of researches as a medical-friendly biomaterial. Originating from natural substances, it has unique features which make it promising for current and future use in human and veterinary medicine. There is research indicating its usefulness in such practical situations as wound management (already registered products). Most interesting is using chitosan as a scaffold for tissue engineering, and therefore faster and complete tissue regeneration. Although it still has some drawbacks, combination with other polymers might reduce them.

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