Bulgarian Chemical Communications, Volume 47, Special issue A (pp. 10 – 15) 2015

Review and comparative analysis of keratin biocomposites with composites based on collagen

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Received August 1, 2014; Accepted January 26, 2015

Keratin and collagen are ones of the most abundant proteins which can be used in a variety of biomedical application due to their biocompatibility and biodegradability. Even though, keratin and collagen biomaterials are very high potential for tissue engineering applications. But, the major disadvantages of the natural biomolecules are their poor mechanical properties. Therefore it must be modified or combined with synthetic polymers. More of the synthetic polymers have good mechanical properties and thermal stability and numerous studies of biocomposites based on collagen or keratin and synthetic polymers are performed. The polyurethanes (PUs) are considered the most promising class of synthetic polymers for *in vivo* studies, and fulfill all the criteria for application to medical practice.

Keratin biomaterials have many various advantages over conventional biomolecules, including unique chemical properties due to the high content of sulfur in their structure, high biocompatibility, having aptitude to intracellular recognition and tendency to self-configure. There have been a lot of studies for the composites based on the keratin and one of the following synthetic polymers: poly(ethylene-oxide) (PEO), polyamide (PA6); poly(vinyl alcohol) (PVA), poly(methylmethacrylate) (PMMA), poly(L-lactic acid) (PLLA), etc., with the application in tissue engineering and drug delivery, in the form of gels, sponges, foams, films, fibers, mats and others. In the literature, however, no investigations for the composites based keratin/polyurethane for biomedical applications.

Key words: biocomposites, keratin, collagen, polyurethane, biocompatibility

INTRODUCTION

Collagen is the most abundant used tissue-derived natural polymer, and it is a main component of extracellular matrices of mammalian tissue including skin, bone, cartilage, tendon and ligament. It possesses low immunogenicity, high absorbance and very good biodegradability and it is suitable for preparing of biomaterials with different properties, composition and form by combination with synthetic polymers [1-25].

Keratin is the most abundant non-food protein being the major component of wool, hair, horns, nails and feather. Biomaterials based keratin are proven promising class of natural polymers due to their typical biocompatibility, biodegradation, chemical resistance, biological activity, higher cell adhesion compared to collagen and the possibility of polymerization in the porous structures [26-45].

The disadvantages of the proteins as biomaterials are: their similarity with substances naturally surrounding; poor mechanical properties; proteolytic modification at temperatures below its melting point; difference in the structure of the macromolecular substances which are derived from different animal sources. Each of these polymers is different from one another (specificity of samples) but also by one of the other tissues (tissue specificity) [6,14,18].

In recent years, hybrid polymeric systems of natural and synthetic macromolecules are used as medical biomaterials. The main biopolymers or called animal-based proteins used in preparation of materials for biomedical applications, are collagen, chitin, chitosan, keratin, silk and elastin [12,13,14,28,45].

The term "composite" means having two or more different parts [14]. Most composites are produced to ensure the mechanical properties such as strength, stiffness, toughness and fatigue resistance combined with other necessary qualities: biological functions and biocompatibility. Polyurethanes (PUs) are synthetic materials, which are obtained from isocyanates and hydroxyl containing components, are considered the most promising class of polymers for *in vivo* studies [4,12,13,18]. The reasons are their

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high mechanical strength, elasticity, aging resistance, tissue compatibility. Another advantage is their biodegradability by resorption. The PUs can be varied in a wide range by the proper selection of components, composition and preparation conditions. The PUs have a lot of functional groups: hydroxyl-, isocyanate-, urethane-, ester-, ether, alofanate-, etc. The collagen or other polypeptides, including the keratin, can react with terminal groups of PUs, which may result in bioactive composites. Consequently composites are based on keratin or collagen and polyurethane, combining the advantages of natural and synthetic materials would be promising biomaterials for tissue engineering, drug delivery and others.

The purpose of this study is to review and analyze the established possibilities for obtaining and application the biocomposites based on keratin/ polyurethane in the medicine and other areas as compared to those based on collagen/ polyurethane.

COLLAGEN AND COMPOSITES BASED ON COLLAGEN/ POLYURETHANE

Collagen is the most abundant protein. It is found in all multicellular organisms in the animal world. About 60% of the total content of protein in living organisms falls on collagen. It is available in large quantities, particularly as waste from slaughterhouses and leather industry. During the 80s of XX century, the collagen became a commonly used biomolecule in many medical applications. From a scientific point of view, which appears to collagen is understandable because it belongs to the so-called "grateful" biological objects for investigation. It can easily be isolated in pure form of insoluble fibers; it has a significant chemical and thermal stability as well as highly ordered structure. Due to its valuable biological properties: biocompatibility and biodegradability, which are well studied, there is a growing scientific interest towards it, especially in the field of bone implant surgery [1,3,11-25].

Collagen and elastin are two of the key structural proteins which have been found in the extracellular matrix of many tissues [20,21]. These proteins are important modulators for the physical properties of any engineered scaffolds, affecting cellular attachment, growth and responses to mechanical stimuli.

Natural polymers such as collagen and elastin are usually insoluble both in water and organic solvents [12,14]. The exception is collagen extracted from tissues of young animals, which is soluble in dilute acetic acid. The solubility of collagen in acetic acid provides the possibility to blend it with other water soluble polymers. Mixtures of collagen with synthetic polymers as well as with other natural polymers has been widely studied as biomedical materials, as have been collagen itself [14,26].

Biocomposites based on collagen, water-soluble synthetic polymers, namely polyvinyl pyrrolidone (PVP); polyvinyl alcohol (PVA); polyethylene glycol (PEG); polyethylene oxide (PEO), prepared in the form of thin films, hydrogels and sponges, have been studied in detail in medical practice. For the preparation of biocomposites are used and other synthetic polymers, namely: polyurethanes (PU); polyglycolic-(PGA), polylactic acid (PLA), poly (DL-lactide-co-glycolide) – PLGA [11-25].

Polyurethanes (PUs) are considered the most promising class of polymers for *in vivo* studies such as fulfills all the criteria for applicability to medical practice. PUs are potentially biodegradable materials, have unique chemistry and processability, but there is some limitation on their application due to lack of biologically active groups [4-10].

Jianjun *et al* [17] developed a flexible, biodegradable scaffold structure for cells transplantation in the form of a composite material based on biodegradable poly(esterurethan) urea and collagen type I. Poly(esterurethan)urea was synthesized by poly(caprolactone), 1,4-diisocyanatebutan and putrescine.

Oprea [18] studied the synthesis and properties of the microporous material based on polyurethane and collagen (0-15 wt.%). Polyurethane is prepared by polyadditional reaction on polyetherdiol with an aliphatic 1,6-hexamethylene diisocyanate and castor oil as the three-functional extender of the polymer chains. The obtained two-phase structure is characterized by excellent mechanical properties, pores with required sizes and biocompatibility, which are important parameters for biomedical applications.

The formation of the first primary compact and secondary porous structure can be explained by the surface gelling mechanism, in which the collagen migrates towards the surface of the composite film and the big size pores are formed at this stage. These pores were randomly distributed and interconnected [14,18]. Pore interconnectivity is due to the presence of smaller pores (up to 50 μm) that are positioned adjacent to the collagen fibers. It also has been observed that fewer small numbers of small pores are present in the PU (polyurethane) phase with a higher content of the collagen. The decomposition temperature in the main stage and mechanical properties decreased with increasing the collagen content. The measurements of the contact angle show that the collagen improves the hydrophilicity of the PU-Collagen composites.

In order to improve the cells adhesion the coatings from collagen onto numerous hydrophilic polymeric scaffolds were applied [19].

The regeneration of damaged or lost tissue requires that certain function reparative cells assemble three-dimensionally around and inside the surrounding scaffold [24]. The nanofibres based on collagen and functionalized thermoplastic polyurethane (TPU/ Collagen) were successfully synthesized by electrospinning technique in order to obtain biomedical material with a porous structure. The results show that electrospun nanofibre composites exhibit features of natural extracellular matrix and can be effectively used as an alternative material for tissue engineering and as functional biomaterials.

The effects of the different parameters on the preparation process of porous structures have been studied in order to obtain optimal polyurethane scaffolds for the cardiovascular engineering.

We synthesized and investigated the composites based on polyurethane (PU) and collagen as a filler in concentrations (5 and 10 wt.%) [25]. The synthesis of the PU was carried out by two-step polyadditional reaction of the polyester polyols and 1,6-hexamethylene diisocyanate (MDI), and 1,4-butanediol as chains extender. Collagen was added *in situ* during the polymerization reaction. *In vitro* tests were conducted of the composites prepared in 1.5 SBF (Simulated Body Fluid) for 7 days under static conditions. FTIR (Fourier Transform Infrared Spectroscopy) and SEM (Scanning Electron Microscopy) methods were used for analysis.

Oprea *et al* [18,20] proved that the appearance of the hydrogen bonds between the collagen and the polyurethane macromolecules which is an indication of crosslinking or hardening of the composite structure. The adsorption bands at 1709 cm^{-1} corresponds to the free and H-bonded C=O groups. The same bands are also observed in our investigations. The intensity of the bands at the composites increases as compared to that of pure PU. The amide and carbonyl regions of spectra provided information for the intermolecular attraction by hydrogen bonding.

The micrographs on *fig.2* show the deposition of hydroxylapatite agglomerates with spherical forms after 7 days stay in SBF. The pores are interconnected in the scaffolds and there is the presence of the micropores in macroporous wall on which they to fulfill the criteria of the scaffold structure.

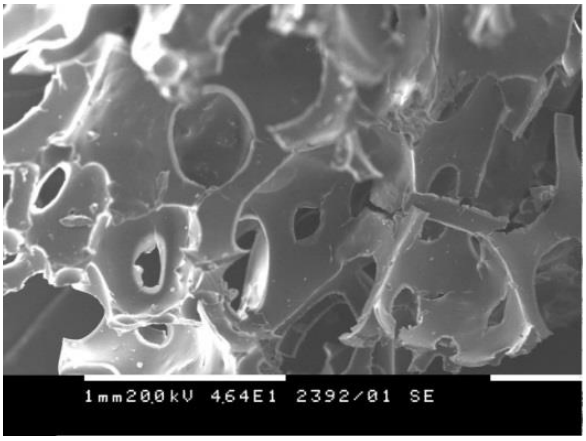

Fig.1. SEM of composites PU/Coll

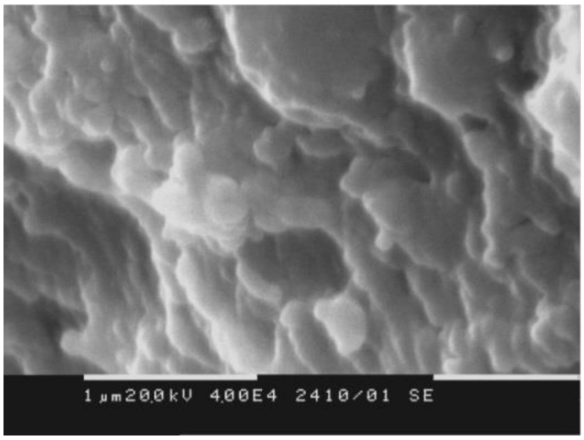

Fig.2. SEM of the PU/Coll after 7 days in SBF

KERATIN AND ITS COMPOSITES

Keratin is one the most abundant animal protein and is a waste from slaughterhouses, the leather and textile industry. It is a major component of hair, horns, nails, feathers, hooves and the stratum corneum of the epidermis. General, which bring together various keratins in one class proteins, is the presence of large amounts of cysteine, and thus the sulfur in its composition. Keratin is characterized by a higher cell adhesion compared to collagen. It has ability to polymerize in the porous structures as collagen and to self-assemble into complex of three dimensional scaffold structures [1,2,12,13,27].

Keratin belongs to the group of fibrous proteins. The extracted keratin can be produced in different forms: sponge scaffolds, mats, films, sheets, gels, micro fibers and bulk materials [26-45].

Wool keratin has been studied for application in cosmetic and tissue engineering for fabrication of sponge scaffolds for cells cultivation due to the close chemical similarity with human skin and hair or has been electrospun with other polymers to produce blend nanofibers with potential applications in different areas, from technical textiles to biomedical commodities [32].

It was limited the use of many biomaterials of natural origin, especially keratin-based products due to their poor mechanical properties. Thus the focus of research shifted to optimize the physico-mechanical properties and the preservation of their excellent biological activity. Several approaches have been considered to control the physical and biological properties, including the addition of natural and synthetic polymers to the keratin compounds and the application of novel preparative techniques to obtain pure keratin films [33,44].

Therefore keratin is suitable basic material for obtaining of hybrid systems with different characteristics, composition and forms, in combination with synthetic polymers. To the keratin is particularly interested in developing new products for the pharmaceutical industry, medicine, cosmetics and biotechnology.

The earliest documented use of keratins for medicinal applications comes from a Chinese herbalist *Li Shi Zhen* in 16th century [26]. A substance made of ground ash from pyrolized human hair that was used to accelerate wound healing and blood clotting called *Xue Yu Tan*, also known as *Crinis Carbonisatus*, was described in his book.

During the years 1905 to 1935, many methods were developed to extract keratins using oxidative-reductive chemistries. These technologies were initially applied to animal horns and hooves, but were also eventually used to extract keratins from wool and human hair.

Advances in the extraction, purification and characterization of keratins, led to exponential growth of keratin materials and their derivatives in the 70s of the last century [26]. The potential uses of keratins in similar applications began to be explored by a number of scientists. In 1982, Japanese scientist published the first study describing the use of keratin coating on vascular grafts as a way to eliminate blood clotting, as well as experiments on the biocompatibility of keratins.

In order to enhance the mechanical properties of the glycerol-containing keratin films by adding the chitosan or keratin experiments were done [35]. The composite films obtained show enhanced anti-thrombo-genetic properties and increased biocompatibility compared with fibroin itself or keratin.

Authors [36] were studied the interaction between keratin and synthetic polymers, namely on the interaction between the poly(ethylene oxide) (PEO) and the keratin film in order to obtain keratin biomaterials with improved properties. These composites can be used as scaffolds supporting the cells growth, dressing materials, membranes for the drug delivery.

It was investigated the intermolecular interactions between keratin and polyamide 6 (PA6) [39] in order to obtain keratin materials with a wide range of applications: from biomedical devices to active water filters and textile fibers.

Various methods for the processing of keratin with synthetic or natural polymers are applied in order to improve its processability in fiber-materials. Materials based on keratin/ PEO have been synthesized by mixing an aqueous solution of keratin and powdered PEO with good mechanical properties [37].

Fibers were obtained from aqueous solutions of the keratin with polyvinyl alcohol (PVA) [38]. The obtained composites showed good mechanical strength, water resistance, and excellent adsorption ability to toxic substances. Therefore with the success can be applied as absorbents for toxic substances, heavy metal ions and toxic gas from the formaldehyde.

Keratin fibers are used as reinforcing filler for the matrix of PMMA (polymethyl methacrylate) [39]. The composites have a high thermal stability and the keratin well distributed in the polymer, which leads to an increase of visco-elastic properties at elevated temperatures.

Keratin fibers are used in composites of polypropylene [43]. Maleic anhydride is grafted to the polypropylene in order to improve the dispersion of the keratin. These keratin materials increase the rate of crystallization of the polypropylene and thus improving its thermal stability.

As a native protein, keratin from wool is used to improve the affinity of the cells to poly (L-lactic acid) (PLLA) [44]. It has been found that the keratin supports interactions between osteoblastic cells and the polymer scaffold.

Wet-spinning techniques have been developed to produce novel keratinous fiber materials with potential use as hygiene articles [42]. Composites are prepared on the basis of keratin derived from feathers and modified bio-cellulose and used

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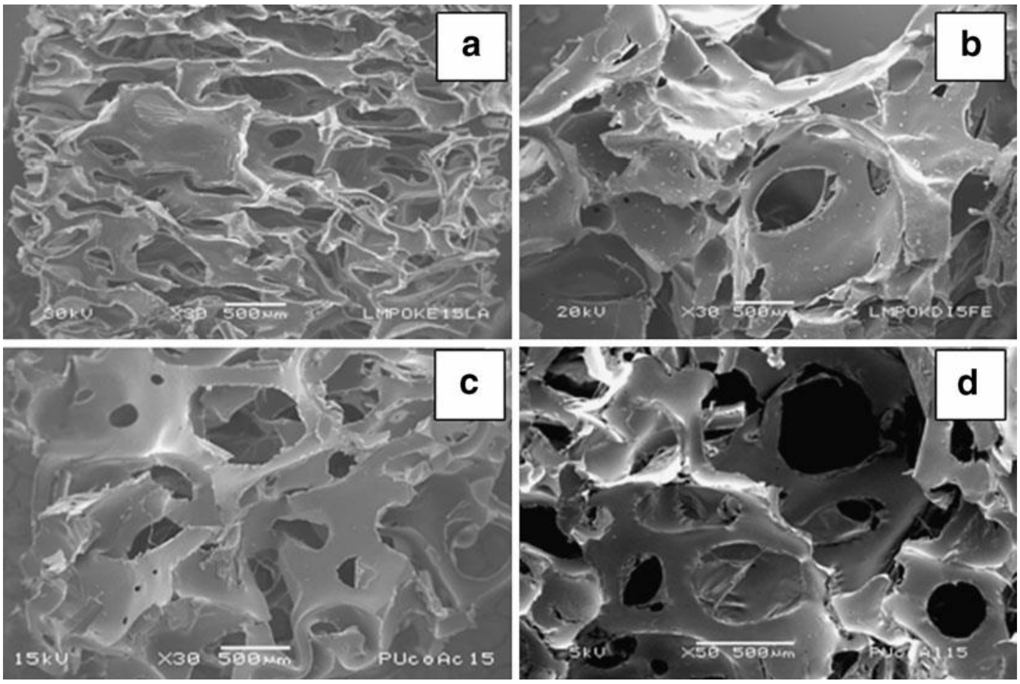

Fig.3. Micrographs of polyurethane-keratin membranes: a) keratin salt, b) dialyzed keratin, c) alkaline biofiber solution, d) acid biofiber solution, all of them at 15 wt.% [40]

to produce fibers, which are characterized by a good sorption properties, higher hygroscopicity and a lower contact angle than the cellulose fibers themselves.

The authors [40] have studied the possibility for use of the porous membrane based on polyurethane/ keratin to remove of hexavalent chromium. Keratin creates active sites for bioabsorption of Cr (VI) and polyurethane plays an important role in supporting the protein

CONCLUSION

Various biomaterials have been developed, some of which serve to support the healing process, acting as implants, others as drug delivery, to increase the adhesion of cells involved in the construction of the extracellular matrix and others. Some of them are based on keratin and synthetic polymers, combining the positive properties of the natural and the synthetic polymer. In the literature there are no reported studies on biomaterials based on keratin/ polyurethane for biomedical applications.

Keratin biomaterials have many various advantages compared to conventional biomolecules, including unique chemical properties due to the high content of sulfur in their structure, remarkable biocompatibility, tendency for self-configuration and internal cell recognition. Since these properties are becoming better studied, monitored and implemented many biomedical applications of keratin biomaterials have made their way in the clinical studies and will undergo revisions and new research in the development of new methods for synthesis of composite biomaterials.

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ПРЕГЛЕД И СРАВНИТЕЛЕН АНАЛИЗ НА КЕРАТИНОВИ БИОКОМПОЗИТИ С ТАКИВА НА БАЗА КОЛАГЕН

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Постъпила на 1 август 2014 г.; приета на 26 януари, 2015 г.

(Резюме)

Кератинът и колагенът са едни от най-често срещащите се протеини, които могат да бъдат използвани в различни биомедицински направления благодарение на тяхната биоразградимост и биосъвместимост. Освен това кератиновите и колагеновите биоматериали притежават висок потенциал за приложение особено в областта на тъканното инженерство. Основен недостатък на природните биомолекули са незадоволителните им механични свойства. Това налага модификация или съвместяване със синтетични полимери, характеризиращи се с добри механични свойства и термична стабилност. Проведени са многобройни изследвания на биокомпозити на база колаген или кератин и синтетитичен полимер. Най-обещаващ клас синтетични полимери за *in vivo* изследвания се смятат полиуретаните, които отговарят на всички критерии за биомедицинско приложение.

Кератиновите биоматериали притежават много различни предимства в сравнение с конвенционалните биомолекули, включително и уникални химични свойства, дължащи се на високото съдържание на сяра в структурата им, висока биосъвместимост, склонност към вътрешно клетъчно разпознаване и склонност към самостоятелно конфигуриране. Има много изследвания на композити на база кератин в комбинация с различни синтетични полимери, а именно: полиетиленоксид (PEO), полиамид (PA6); поливинилацет (PVA), полиметилметакрилат (PMMA), поли(L-млечна киселина) (PLLA) и др., с приложение в тъканното инженерство и за доставка на лекарства, под формата на гелове, гъби, пени, филми, влакна, подложки и др. В литературата обаче липсват изследвания специално на композити на база кератин/ полиуретан за биомедицински приложения.