THE COLLAGEN IN REGENERATIVE MEDICINE – PRESENT STATUS AND SOME MOVING TRENDS

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Abstract

In the new paradigms of regenerative medicine, the use of materials in contact with biological materials (cells, tissues/organs, physiological fluids and biomolecules) is a current illustration of the need of interdisciplinary scientific approaches that combine the most recent advances in materials science and technology, basic sciences and life sciences.

In tissue engineering, matrices are developed for cells support, promoting their differentiation and proliferation towards the formation of a new tissue.

Such strategies allow for producing hybrid constructs that can be implanted in patients to induce the regeneration of tissues or replace failing or malfunctioning organs. Different materials have been proposed to be used in the processing of scaffolds, namely biodegradable polymers.

In this review, the natural-based materials of collagen that have been proposed to be used in tissue engineering strategies and isolation methods of collagen will be overviewed.

Key Words: tissue engineering, biopolymers, collagen type I, isolation methods, 3D-matrix.

Rezumat

Colagenul în medicicna regenerativă – present şi perspective

În paradigma nouă a medicinei regenerative utilizarea materialelor care vin în contact cu materialele biologice (celule, ţesututi, organe, lichide fiziologice şi biomolecule) necesită studii ştiinţifice interdisciplinare, care combină cele mai recente performanţe în ştiinţa şi tehnologia materialelor, ştiinţele de bază şi a vieţii.

În ingineria tisulară sunt dezvoltate matrice pentru suportul celulelor, cu proprietăţi de a induce diferenţierea şi prolifirarea în direcţia formării noului ţesut.

Se necesită obţinerea construcţiilor hibride care pot fi implantantate la pacienţi pentru a induce regenerarea ţesuturilor sau înlocuirea oreganelor nefuncţionale sau cu funcţia redusă.

În acest articol de sinteză sunt descrise şi analizate materiale de origine naturală, anume a colagenului, metodele cunoscute de izolare şi purificare a colagenului ce sunt propuse pentru a fi utilizate în perspectiva ingineriei tisulare.

Cuvinte cheie: Ingineria tisulară, colagen tip I, metode de izolare, matrice 3D.

Introduction

Tissue engineering is a multidisciplinary field which involves the application of the principles and methods of engineering and life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes that restore, maintain or improve tissue function [1]. The goal of tissue engineering is to surpass the limitations of conventional treatments based on organ transplantation and biomaterial implantation [7].

It has the potential to produce a supply of immunologically tolerant “artificial” organ and tissue substitutes that can grow with the patient. This should lead to a permanent solution to the damaged organ or tissue without the need for supplementary therapies, thus making it a cost-effective treatment in the long term [8].

Natural-based polymeric sistems

The design and selection of a biomaterial is a critical step in the development of scaffolds for tissue engineering. Generally, the ideal biomaterial should be non-toxic, biocompatible, promoting favourable cellular interactions and tissue development, while possessing adequate mechanical and physical properties [16, 29].

In addition, it should be biodegradable and bioresorbable to support the reconstruction of a new tissue without inflammation [4]. On the other hand, novel concepts of tissue engineering are

imposing new and more specific requirements on macromolecular components. Living organisms are able to synthesize a vast variety of polymers, which can be divided into major classes according to their chemical structure:

I Polysaccharides – starch, cellulose, agar, chitin, dextran, gellan gum, hyaluronic acid, glycosaminoglycans;
II Complex proteins – collagen, fibronectin, fibrin;
III Polyesters – polyhydroxyalkanoates;
IV Natural inorganic materials – e.g. hydroxyapatite, tricalcium phosphate.

Biomaterials play a crucial role in tissue engineering by serving as 3D (three dimensions) synthetic frameworks (commonly referred to as scaffolds, matrices, or constructs) for cellular attachment, proliferation, and in growth ultimately leading to new tissue formation [3].

Nowadays, with the advances in biotechnology, natural polymers can be obtained by the microorganisms fermentation [5] or produced *in vitro* by enzymatic processes [1, 28]. However, the largest amount is still extracted from plant [7] and animals [8] or from algae [2] .

Synthetic polymers are widely practiced, for example aliphatic polyesters such as polyglycolic acid (PGA), polylactic acid (PLLA), their copolymers (e.g. PLGA) and polycaprolactone (PCL). These polymers are the most commonly used in tissue engineering. The degradation products of these polymers – glycolic and lactic acid – are present in the human body and are removed by natural metabolic pathways [4, 5].

General physical-chemical proprietis of collagen

Collagen is one of the most abundant proteins in mammalians and strongly conserved throughout evolution. It constitutes one third of the human proteome and comprises three quarters of the dry weight of human skin. It is widesperide as a major structural component in animal body such as in bones, cartilage and skins [2, 22]. More and more studies have shown that, in addition to the structural function, collagens can induce or regulate many cellular functions and processes such as cells differentiation, motion, communication and apoptosis [17, 18, 27].

Collagen is the major component in the extracellular matrix and more than 20 genetically various isoforms have been identified, and type I, II and III collagen are the most abundant and investigated for biomedical applications [25, 32]. It is the major structural component in connective tissues such as tendon, skin and blood vessels. Type I collagen has been discribed as a potential candidate for use as a natural scaffold for tissue engineering and reconstructive medicine [32-36].

The molecular mechanism for the biosynthetic assembly of collagen is still of great interest. Type I collagen is trimeric [(α1)2β2] and exists as triple helix. The helices have the typical trimer repeats of Gly-X-Y for collagen. Iminoacids - proline or hydroxyproline constitute about 1/6 of the total protein sequence. Collagen type I usually forms fibrils with a length of 300 nm and a fibrillar diameter of up to 1000 nm [33, 34]. The structure and assembly of the triple helix have been extensively studied for more than 40 years. The spontaneous formation of triple helices from isolated α1 and α2 polypeptides *in vitro* has been studied extensively [25] and recent studies using synthetic model peptides [26, 29] show the self-association or self-assembly of these peptides to a native-like triple helix structure characteristically found in collagen fibrils. However, the studies using naturally derived collagens have been strongly hindered due to the high molecular weight of native collagen and limited understanding of the function of the collagen peptides, in particular the N- and C-terminal regions [19].

The application of Type I collagen is increasing continuously, such as in tissue engineering as natural matrix. However, different non-understood features of collagen, including its assembly, folding, posttranslational modifications, export and cellular function persists. This understanding is particularly hampered by the isolation process, which commonly uses acetic acid-extraction. Moreover, most structural studies have been carried out using model peptides leading to limited understanding of the collagen structure and assembly [26, 27, 31].

Collagena isolation and purificatipon

Through the extreme diversity of tissues and types of collagen it is difficult to develop a standard method of extraction for all types of collagen and from different tissues. Covalent intermolecular interactions in structure of collagen, which account increases in time, determine almost full insolubilization in usually utilized solvents for proteins [2, 35-36].

Depend of specific protein solvent of it is accepted the next types of soluble collagen :

- neutral salt-soluble collagen
- acid-soluble collagen
- enzymatic-soluble collagen

It has been known for a long time that collagen can be isolated by extraction in neutral salt or low ionic strength acidic solutions. In neutral salt solutions, e.g. 1 M NaCl, 0.05 M Tris, pH 7.5, collagen can be solubilized in solution. The efficiency of the extraction is also increased with increased salt concentration. However, in normal tissues the proportion of neutral saltsoluble collagen is very small so that the final yield is very low [35-36].

Moreover, a variety of tissue proteinases may also be present and active in this solubilizing system. To minimize enzymatic cleavage, numerous proteinase inhibitors (such as EDTA) should be present during the extraction [34].

Another widely used method is solubilization with diluted organic acid e.g. 0.5 M acetic or citric acid, pH 3. It has also been described that lowering the pH to 2.5 in the presence of EDTA effectively inhibits degradation. Clearly, this method has a higher capacity to solubilize collagen than neutral salt but is still limited to younger non cross-linked tissues [24].

Thus an acid-based approach was developed which contains a pepsin digestion as the first extraction step. However, the amount of pepsin sufficient for collagen solubilization is tissue dependent. Unfortunately, pepsin can also cause cleavage of collagen [19, 35].

Nevertheless, the method using acetic acid to extract collagen from tissues is well established and the most widely used in research and in industrial production of collagen. Although this extraction was standardized more than 40 years ago remains still two major problems. First, the definition of collagen solubility is still ill-defined due to cross-linking mediated aggregation, so that the reproducibility of the collagen preparations is poor. Secondly, the collagen peptides especially the short non-helical regions of collagen are susceptible to proteolysis/hydrolysis during the isolation [30]

In addition to these two problems, the duration required to solubilize collagen from tissues is normally between 1-3 weeks, with high protein loss and partial degradation of the collagen peptides [20]. For this reason the utility of the acidic-extracted collagen is limited, as the isolated material must be stored in cold acetic acid solution or dried. The maximal concentration of collagen obtainable is also limited to 10 mg/ml as estimated by wet weight and also by amino acid composition. Unfortunately, the protein determination is also limited by the non-applicability of common methods such as Lowry or Bradford [8].

To overcome these disadvantages, some researchers have tried to extract collagen using 8-10 M urea followed by centrifugations and different chromatographic steps using carboxymethyl-cellulose (CM-cellulose) or similar ion exchange materials [2]. However, the attempts which focused on the isolation of α1-chain from Type I collagen or procollagen have reported extensive precipitation, irreversible denaturation and enzymatic cleavage during isolation even in 8 M urea [3]. All of these reports emphasizies the poor quality of the obtained collagen. This has resulted the infrequent use of the urea-extraction procedure.

It is required the purification of collagen to eliminate the antigenic components of the protein. These are the telopeptide regions of collagen type I that can be most efficiently treated by enzymatic digestion. Pepsin is a widely used enzyme for the elimination and digestion of this immunogenic peptide [2, 15]. As an example, rat tendon collagen type I was extracted and purified in 0.5mg/ml Pepsin in 0.5M acetic acid for 24 hours [15]. However, complete immunogenic purification of non-human proteins is difficult, which may result in immune rejection if used in implants. Impure collagen has the potential for xenozoonoses, the microbial

transmission from the animal tissue to the human recipient [3]. However, although collagen extracted from animal sources may present a small degree of antigenicity, these are considered widely acceptable for tissue engineering on humans [4].

Applications of collagen type I

As mentioned above collagen is a major component of all tissues and has a variety of structural functions, so that this protein is regarded to be one of the best candidates for a wide range of medical applications. There is a long history for the usage of collagen for numerous applications including drug delivery systems [10], scaffold in tissue engineering and regenerative medicine [3, 13, 29]. In comparison to other biomaterials, naturally derived collagen shows excellent biocompatibility and safety due to its high abundance in all vertebrate animals and high biodegradability [12]. In addition, collagen also exhibits very low antigenicity [15].

In the last two decades, many researchers have shown that collagen has not only support function but is also involved in many other cellular processes, including regulation of cell motion, cell proliferation and cell apoptosis [11]. Also in some biological research areas Type I collagen has been applied as a coating for culture dishes or as a scaffold for microbiological adherence and invasion test systems [14, 23].

Solubilization of collagen can give us a nice opportunity the wide introducing in the tissue engineering. It could be in next forms: matrices, powder, sponge, fibres or filaments.

One of the principle of tissue engineering is involving the growing of the relevant cell(s*) in vitro* into the required three-dimensional (3D) organ or tissue. But cells lack the ability to grow in favoured 3D orientations and thus define the anatomical shape of the tissue. Instead, they randomly migrate to form a two-dimensional (2D) layer of cells. However, 3D tissues are required and this is achieved by seeding the cells onto porous matrices, known as scaffolds, to which the cells are attached and made colonies [2]. The scaffold therefore is a very important component for tissue engineering [3].

Despite enormous advances in the fields of materials science and cell biology, major challenges remain for engineering materials that control and regulate cellular behaviour and generation of tissues that can substitute for at least some functions of human organs. Because cells isolated from organs can not spontaneously reassemble into functional tissues by themselves, in most approaches of tissue engineering, synthetic materials are used to help the cells to get properly organized [6, 9].

The first generation of cellular scaffolds has been used successfully as a substitute for the skin [21]. For these skin equivalents, there were at least two different types: in one skin equivalent that recently obtained FDA approval (DermagraftTM), dermal fibroblasts are suspended in a polymer mesh [1]; an another product (ApligrafTM), fibroblasts are seeded in a collagen gel that is then coated with a layer of human epidermal cells [4]. The main problem is that the porous structure of collagen spheres show pores about 200-300 μm which are closer to those of a 2D-matrix rather than the size observed for a typical 3D-matrix in situ [15].

Conclusions

Recent studies in cell biology, nanotechnology, and computation give more new insights especially the understanding that physical proprities in addition with chemical can regulate cell signalling and gene expression. Due to the extreme importance of biocompatible matrices for tissue engineering and application in medical technology, the availability of native collagen should be studied by refining the extraction procedure of collagens.

One of the goals is to reexamine the quality of Type I collagen after acid-extraction, using pepsin enzymatic extraction and urea-extracted collagen. It is very important that elaborated type of tissue processing give us fully or partially denaturated collagen, or solutions with characteristics similar to native collagen.

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INFLUENȚA REMEDIULUI BioR^{Se} ASUPRA ACTIVITĂȚII SISTEMULUI ANTIOXIDANT ÎN FICAT ÎN PROCESUL DE REGRESIE A CIROZEI HEPATICE EXPERIMENTALE

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Summary

The influience of the remedy BioR^{Se} on the activity of anti oxidant system in liver during the recovery from experimental hepatic cirrhosis

It was investigated the antioxidant state in the hepatic parenchyma at different stages of recovery from hepatic cirrhosis and the influence of the remedy BioR^{Se} on the antioxidant system in liver. It was determined the activity of the most important enzymes of the antioxidant system – catalase, glutathione-reductase and glutathione-S-transferase. It was determined that the administration of BioR^{Se} during the cirrhosis regression has a beneficial effect on the restoration of the antioxidant properties of the liver parenchyma.

Rezumat

În lucrare se apreciază statutul antioxidant al parenchimului hepatic la diferite etape ale regresiei cirozei hepatice şi influența remediului BioR^{Se} asupra stării sistemului antioxidant în ficat. A fost determinată activitatea celor mai importante enzime ale sistemului antioxidant -