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Preparation and characterization of an advanced collagen aggregate from porcine acellular dermal matrix

Xinhua Liu[a,b], Nianhua Dan[a,b], Weihua Dan[a,b,*]

[a] Key Laboratory for Leather Chemistry and Engineering of the Education Ministry, Sichuan University, Chengdu, Sichuan 610065, China
[b] Research Center of Biomedical Engineering, Sichuan University, Chengdu, Sichuan 610065, China

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ABSTRACT

The objective of this study was to extract and characterize an advanced collagen aggregate (Ag-col) from porcine acellular dermal matrix (pADM). Based on histological examination, scanning electron microscopy (SEM) and atomic force microscope (AFM), Ag-col was composed of the D-periodic cross-striated collagen fibrils and thick collagen fiber bundles with uneven diameters and non-orientated arrangement. Fourier transform infrared (FTIR) spectra of pADM, Ag-col and Col were similar and revealed the presence of the triple helix. Circular dichroism (CD) analysis exhibited a slightly higher content of α-helix but inappreciably less amount of random coil structure in Ag-col compared to Col. Moreover, imino acid contents of pADM, Ag-col and Col were 222.43, 218.30 and 190.01 residues/1000 residues, respectively. From zeta potential analysis, a net charge of zero was found at pH 6.45 and 6.11 for Ag-col and Col, respectively. Differential scanning calorimetry (DSC) study suggested that the T*d* of Ag-col was 20 °C higher than that of Col as expected, and dynamic mechanical analysis (DMA) indicated that Ag-col possessed a higher storage modulus but similar loss factor compared to Col. Therefore, the collagen aggregate from pADM could serve as a better alternative source of collagens for further applications in food and biological industries.

1. Introduction

Collagen is the most fundamental component in the extracellular matrices of connective tissues in mammals and constitutes about 30% of the total proteins [1]. In recent years, collagen has achieved much more attention and been widely used in food, cosmetic, biomedical and pharmaceutical industries due to its superior comprehensive nature [2]. Nevertheless, evidence to date indicates that from the perspective of collagen's biosynthesis in vivo, once collagen molecules are biosynthesized, they would be discharged through the cytomembrane and further self-assemble into collagen aggregates spontaneously in the extracellular matrix [3]. Therefore, collagen is most commonly existed in animals as long, slender generally cylindrical fibrillar aggregation structures with tapered ends that are most easily recognized by a 65–67 nm axial periodicity [4]. These supra-fibrillar structures play a pivotal role in scaffolding structures of the body tissues and are also tightly associated with the bespoke physicochemical, mechanical and biological properties of the tissues from the nanoscopic to macroscopic length scales [5]. Additionally, many biopolymers (such as elastin, fibrillin, polysaccharide, etc) could selectively attach to the interface of these suprabundle structures. These elastic constituents complement the stiff collagen fibers and endow the fickle functional features of the tissues [5–7].

The biosynthesis and assembly of collagen into its hierarchical fibrillar architectures in vivo is very complicated, involving numerous intracellular and extracellular steps. Briefly, it includes the following main phases at the supramolecular level: firstly, the collagen molecules with a length and width of about 300 nm and 1.5 nm, respectively, are generated by the assembly of three polypeptide chains [8,9]; then the collagen molecules would further assemble by a parallel staggering into microfibrils; whereafter, a bunch of collagen microfibrils are oriented not only longitudinally but also transversely and horizontally to form collagen fibrils with a wide diameter distribution ranging from 50 to a few hundred nanometers [10–12]. Therefore, these collagen fibrils are the elementary building block in the collagen-rich tissues. And collagen fibril is a complex supramolecular aggregate and its most typical structural feature, which has long been the focus of attention, is the repeating banding pattern with a so-called D periodicity of approximately 65 nm, i.e. a gap and a short *N*- and C-terminal over-

* Corresponding author at: Key Laboratory for Leather Chemistry and Engineering of the Education Ministry, Sichuan University, Chengdu, Sichuan 610065, China.
E-mail address: danweihua_scu@126.com (W. Dan).

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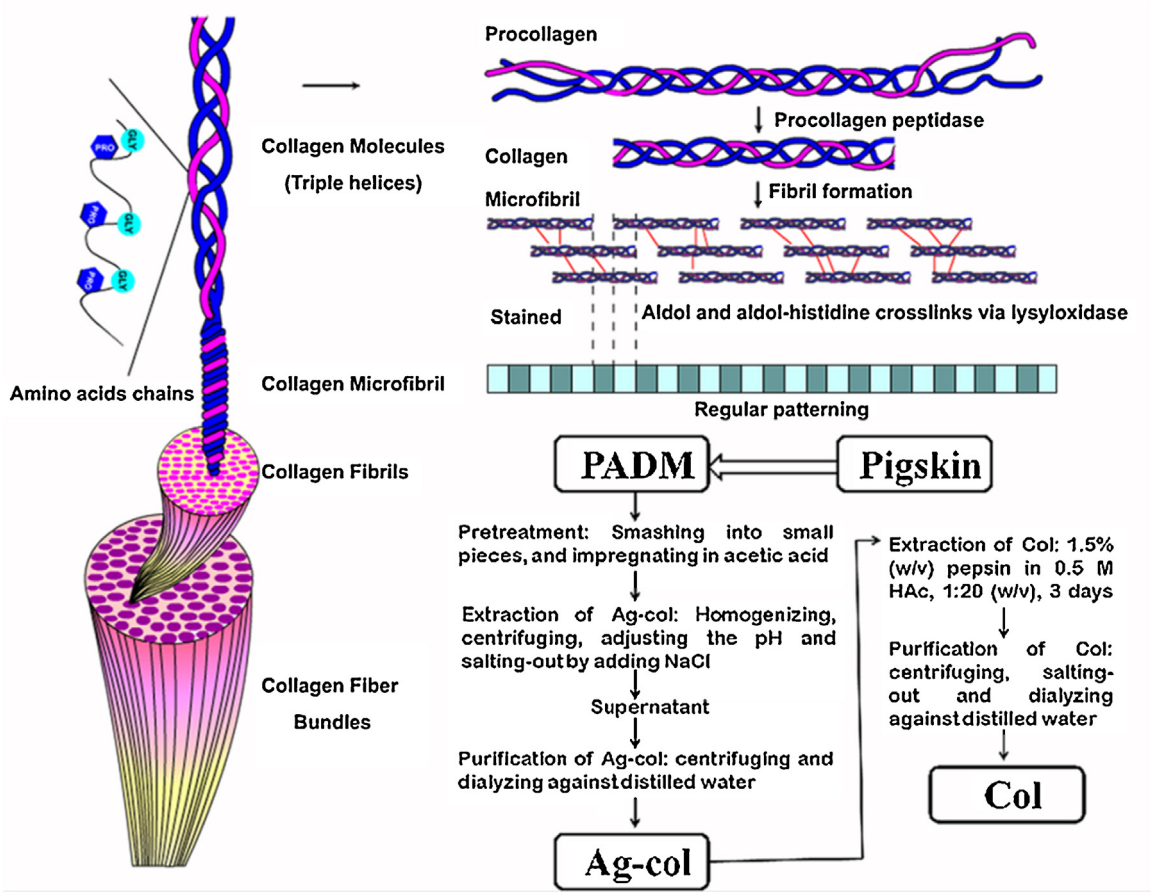

Scheme 1. Schematic diagram showing the isolation procedures of Ag-col and Col, and the hierarchical structure of the collagen aggregates.

lap area within the staggered array of collagen molecules in the fibril [13,14]. More to this point, the covalent cross-linking, electrostatic and hydrophobic interactions, as well as hydrogen bonds and hydration forces among collagen molecules have a direct influence on the repeated periodical D-banding [15,16]. Besides, a group of these fibrils would further accumulate to form more complex collagen fiber bundles that constitute macroscopic tissues. Noted that the diameter, length and microstructure of collagen fiber bundles vary greatly from one tissue to another in one body and even from one area to another in one tissue, which are very crucial to the functional properties of the tissues [17,18]. It is well known that the molecular structure of a substance could predetermine its relevant properties accurately. Likewise, the fundamental properties, especially their thermal-stability and mechanical properties of collagen-based materials with different hierarchical architectures from molecules to mature tissues present an obvious distinction [19,20]. Therefore, hierarchical organization at every structural level of collagen materials allows for the intervention and interplay of a series of design features, which is very significant for its particular applications [21].

Over the few decades, the regenerated collagen fibrils or microfibrils has been reconstituted by the fibrillogenesis of collagen in vitro [22,23]. Evidence to date indicates that when the pH value is adjusted to around 7.0 and the temperature is raised to around 37 °C simultaneously, collagen molecules would assemble into the banded collagen fibrils or microfibrils with uncontrollable diameters and length spontaneously [24]. It is also reported that many parameters, such as buffer compositions, intactness of the N- and C-telopeptides, the presence of macromolecules other than collagens and the order of the initiating procedure of fibril formation, etc. [24,25], could influence and regulate the microstructure and features of the reconstituted fibrils, which are very important for fine-tuning their properties. From the foregoing, the diameter and length of the reconstituted fibrils or microfibrils formed by self-aggregation of collagen molecules have an apparent diversity along with their preparation conditions, which are further closely related to their performances [26]. Moreover, in vitro the forces between reconstituted collagen fibrils or microfibrils are so infirm that the fibrils could not further self-assemble into the more macroscopic collagen fiber bundles. Therefore, few literatures have reported the preparation and properties of collagen fiber bundles hitherto, which may be an ideal collagen-based material for food and biological applications.

Porcine acellular dermal matrix (pADM) has been received much more attentions on account of its specific three-dimensional (3D) architecture that mimics the biological characteristics of a native extracellular matrix (ECM) [27]. So far, a great deal of emphasis has been put on its tissue reconstruction as a tissue engineering scaffold [28] due to its low immune activity, negligible specific allogeneic cellular immune response, the non-specific foreign body reaction against tissue transplantation and superior biological properties [29,30]. Based on our previous literature [27], the basic building blocks of pADM are collagen fibrils and collagen fiber bundles, which may enlighten us to develop a novel and convenient approach to prepare a supra-fibrillar compound made up of collagen fibrils and collagen fiber bundles with the similar superior comprehensive nature of pADM. The emphasis of this study is to prepare and evaluate an advanced hierarchical collagen aggregate (Ag-col) composed of collagen fibrils and collagen fiber bundles, and further provide a simultaneous comparison of collagen (Col)