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Review

Soluble eggshell membrane: A natural protein to improve the properties of biomaterials used for tissue engineering applications

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A B S T R A C T

Extracellular matrix (ECM) acts as an instructing template for the cells contained in tissues. It plays a vital role in regulating cellular behavior by holding and interacting with various growth factors and signaling molecules. The ECM materials are either directly derived from a natural origin, or synthesized mimicking the natural ECM. In this review, we have addressed the ECM derived from eggshell membrane (ESM). The development of porous structures from natural biopolymers, such as ESM holds a number of advantages for tissue engineering applications. By using ESM in tissue engineering application, the cells attach and function to make a required tissue. Thereafter, the scaffold provides mechanical support as well as a platform for cellular interaction, hence, forming a fully functional tissue. The present review summarizes the structure-function relationship of ESM and advancement in its processing methods; the contribution of its soluble form (soluble eggshell membrane protein, SEP) in the development of promising hybrid biomaterials; and the recent advancement of their applications. In addition, this comprehensive review highlights the use of ESM for guided tissue regeneration; promising future applications of SEP in tissue engineering and regenerative medicine.

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Contents

Abbreviations: ECM, extracellular matrices; EDTA, ethylene diamine tetra-acetic acid; ES, eggshell; ESM, eggshell membrane; GTR, guided tissue regeneration; HA, hyaluronic acid; HAp, hydroxyapatite; HCl, hydrochloric acid; JCT, joint and connective tissues; NEM®, commercial Natural Eggshell Membrane; OPN, osteopontin; PSC, pepsin-solubilized collagen; PVA, poly-(vinyl alcohol); RGD, Arginine-Glycine-Aspartic acid (AAs trimer); SEP, soluble eggshell membrane protein.

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1. Introduction

Functional tissues are not only the aggregates of living cells but also complex meshwork of organized non-living extracellular matrices (ECM). ECM provides the structural support for cellular organization and also determines critical *in situ* cellular behavior through cell-matrix interactions [1]. Subsequently, ECM and cells together determine the unique architecture and characteristics of tissues and organs as a whole. Successful tissue regeneration is associated with cell adhesion to ECM *via* integrins that modulate intracellular signaling events [2]. Additionally, ECM plays significant role in interacting with various growth factors and signaling molecules to regulate cellular behavior demonstrated during cell adhesion, proliferation, differentiation and migration. ECM proteins are generally categorized as: collagen, structural glycoproteins, proteoglycans, and elastin [3]. A number of naturally derived polymers show similarity with ECM (*e.g.*, hyaluronic acid, fibrin glue, silk fibroin and chitosan) for tissue regeneration applications.

Eggshell membrane (ESM), a protein-based fibrous tissue that lies in between the mineralized eggshell (ES) and the egg white to provide protection against bacterial invasion shows close resemblance with ECM constituents [4]. The utility of ESM has long been undervalued, because it was considered as a waste material. However, the research groups have made significant progress in studying this biomaterial because of its unique properties [5–17]. In this context, the porous structure of ESM holds many advantages for biological applications as ESM is easily available, biocompatible, contaminant free, green material that can easily be functionalized as such or after modification [18].

Recently published review articles on ESM discuss eggshell waste as a whole (both ES and ESM) [19,20] and the physical structure of ESM and its various applications [21]. In contrast, the present review summarizes: (i) the structural components of ESM and their correlation with suitable tissue engineering applications, (ii) the traditional and patented methods for separation and processing of ESM as a biomaterial, and (iii) the recent applications of ESM for tissue regeneration.

2. ESM in traditional and modern medicine

For many centuries, ESM has been used for healing the injuries as mentioned in the pharmacopoeia of Chinese medicine by Bencao Gangmu [18]. ESM has also been an important ingredient of “phoenix cloth” as dressing material for healing the burn, decubitus ulcer, corneal ulcer, and tympanic perforation in Chinese traditional medicine [19]. Also, few published reports indicate the applications of ESM as a dressing material for healing burns, ulcers and eye injuries [20–22]. In Japan, Sumo wrestlers still apply ESM as a natural medicine on the injuries [18]. Although these evidences indicate the wound healing property of ESM, the molecular mechanism is still yet not known.

Osteoarthritis, the most prevalent form of arthritis, affects millions of adults around the globe and nearly 27 million adults in the U.S. [23]. The conventional treatment with analgesics and non-steroidal anti-inflammatory drugs (alone or in combination) has low efficacy and significant side effects [24]. A patented ESM product, Natural Eggshell Membrane (NEM®), offers a novel diet complementary for maintaining healthy joint and connective tissues [25].

Robinson and King reported for the first time the role of ESM in eggshell formation in 1963 [26]. The contribution of the hyaluronic acid content of ESM in water retention and anti-bacterial activity was first proposed by Osuoji in 1971 which was later demonstrated by several groups of researchers [27–29]. The pioneer work of Wu et al. in 1995 is the first report which indicates the applications of ESM as a biomaterial owing to its chemical composition and structural characteristics [30].

3. Structure and components of ESM

ESM is a double-layered insoluble sheet which lies in between ES and egg white. It is functionally equivalent to ECM in avian egg development acting as a natural scaffold for biomineralization during the egg shell formation [31–33]. It is interesting to note that the entire ES biomineralization takes less than 24 h [34]. The mineralization process for ES development is among the fastest biological processes known

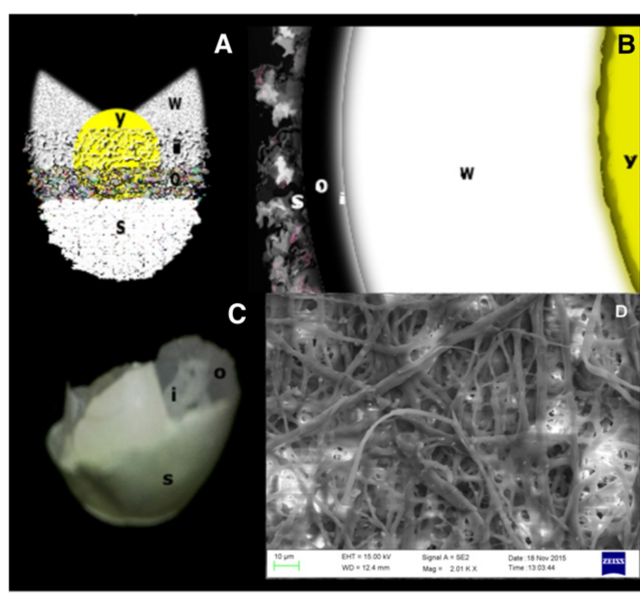

Fig. 1. Schematic presentation of the different components of whole egg (A) and egg shell membrane (B). The parts can be clearly distinguished visually (C). The scanning electron microscopy images show the embedded proteins in the bed of collagenous meshwork (D) [39–41] [s = ES; i = inner ESM; o = outer ESM; w = egg white; y = egg yolk; scale bar = 50 μm (D), 10 μm (E)].

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