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Tilapia fish collagen: Potential as halal biomaterial in tissue engineering applications

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Abstract

Collagen is a natural bioactive polymer widely utilized in tissue engineering applications due to its biocompatibility and biodegradability. Collagen derived from mammalian sources such as porcine and bovine is commonly used as biomaterials. However, due to religious concerns, the halal status of collagen must be put into consideration. Since most of the mammalian collagen is hampered by its haram origins, marine collagens are widely investigated as alternatives for mammalian collagen in tissue engineering applications. Even though the marine collagens are safe and easy to extract, these sources of collagen are hindered by their low denaturing temperature. Tilapia fish (*Oreochromis niloticus*) has long been studied for its potential to substitute mammalian collagen for biomedical purposes due to its higher thermal stability compared to other marine sources. We herein review the potency of tilapia collagen as a biomaterial for tissue engineering applications. In this review paper, we mainly focus on the application of tilapia collagen in the skin, bone/dentin, neural and corneal tissue engineering.

Keywords: Tilapia fish collagen, tissue engineering, halal biomaterial, biomedical.

Introduction

In Islam, halal is defined as permissible (not forbidden by Shariah), whereas haram is defined as not permissible. Halal is no longer considered a mere Muslim's responsibility, but it is considered a way of life for Muslims as well as non-Muslim worldwide. The halal status is not limited to only dietary intake, but it covers a lot of other areas such as cosmetic, pharmaceutical, and medical device products. In 2019, the Department of Islamic Advancement of Malaysia (JAKIM) has expanded halal certification for medical devices, and the standard was published as "*MS 2636 2019 Halal Medical Device-General Requirement*" by Standards Malaysia due to demand

in proposals for specific new products, changing in the classification of medical products and the use of critical ingredients in manufacturing medical devices.

Collagen is known as a critical ingredient in the development of various medical devices including devices derived from tissue engineering (TE) technology due to its nature of origins. Traditionally, most of the collagen is extracted from mammalian sources such as porcine and bovine. However, these sources are hampered by limited applications due to religious concerns (halal status) and the risk of diseases such as bovine spongiform encephalopathy and aphthous fever disease [1]. Therefore, a variety of marine sources were identified as a safe source of collagen and now replacing mammalian collagen for TE purposes. Moreover, the extraction of collagen from the fish by-products would lessen the environmental impact created during the decomposition process and gives an added value to these wastes. However, unlike collagen from terrestrial sources, that of aquatic origin have few disadvantages in terms of sources dependent composition variation and low denaturation temperature [2]. Thus, improvement of physicochemical and biological properties of marine collagen are required so that they can be effectively employed as scaffolds for biomedical applications.

Tilapia fish (*Oreochromis niloticus*) is one of the main fish groups used to develop biomaterials for TE due to its higher denaturation temperature than other marine groups. Over the years, more research has proved the potential of the collagen extracted from skins, scales, and bones of tilapia in substituting mammalian collagen for TE purposes. We herein review the potency of tilapia collagen as a biomaterial in TE applications.

Tilapia fish collagen

The tilapia fish species are native to Africa and Middle East and now has been cultivated and crossbreed in almost all tropical climate countries and subtropical regions. Tilapia species has become the most important food fish and was known as aquatic chicken due to their fast growth rate, adaptability to a wide range of environment conditions, high rate of reproducibility, easy feed and processing [3]. Oreochromis is the most common genus being cultivated and crossbreed producing hybrids. Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), and tilapia from Mozambique (*Oreochromis mossambicus*) are the common tilapia species being cultivated in most of the countries. During industrial processing, approximately 60%-70% of by-products are being produced, including skin, scales, and bones [4]. These parts are rich in collagen and other bioactive molecules. Most of the time, collagens are extracted from Nile tilapia (*Oreochromis niloticus)* species.

Tilapia fish collagen – applications in TE

Tissue injury or organ failure due to severe disease or trauma becomes a major healthcare problem. The available options such as tissue or organ transplantation are limited by the accessibility of a compatible donor and could be very costly [5]. Therefore, TE, an integration of biological science and engineering to regenerate biological substitute for repairing or replacing a damaged tissue or organ, gives a better alternative. TE involves three components; cells, scaffold (3D polymeric matrix), and growth factors [6]. Among these three components, scaffold acts as an important medium for restoring, maintaining, and improving tissue function [7]. Scaffold plays its role in tissue repair and regeneration by providing an appropriate platform, allowing

the essential supply of numerous factors related to survival, proliferation, and differentiation of cell [8,9]. Thus, the scaffold requires particular characteristics such as it must be biocompatible and biodegradable, possesses mechanical properties comparable to the replaced tissue, and support cell attachment and growth [10]. Most of all, it should mimic the ECM in terms of the morphological structure and chemical composition for the cell attachment, proliferation, and differentiation to be occurred [11]. The selection of biomaterials to construct 3D scaffold must be carefully carried out as it has direct influences on cellular behaviors.

Skin tissue engineering

Extreme loss of skin function and structure due to injury and disease may lead to physiological disturbances and subsequently major disability or even death. Current advances in TE catalyze the development of improved cultured skin tissue substitutes. Tissue-engineered skin substitutes for wound healing have progressed enormously over the last couple of years. There are several skin scaffold types such as porous, fibrous, hydrogel, microsphere, composite and acellular [12]. Synthetic and highly biocompatible natural materials have been used to develop skin substitutes and become alternatives to traditional wound-healing strategies and tissue regeneration.

In the field of skin TE, collagen-based skin substitutes are effective in accelerating wound healing by supporting a suitable environment for fibroblast and keratinocyte proliferation [13], [14]. The most commonly utilized forms of collagen-based biomaterials for wound healing and TE purposes are the fibril-forming collagen [15]. In this fibrillar collagen, fibrils are formed from the assembly of tropocollagen triple helices, which then agglomerate to form fibers [15]. Numerous studies have reported the use of tilapia fish collagen in different types of collagen-based scaffold formulations for wound and burn to repair, including collagen-based sponges, electrospun collagen nanofibrous, collagen composite film, and drug-loaded collagen hydrogel. The presence of tilapia collagen in the composite scaffolds enhanced several properties of the skin scaffolds. For instance, composite porous scaffolds made of chitosan, tilapia fish skin collagen, and glycerine were proved to facilitate fibroblasts and keratinocytes infiltration, adhesion, proliferation, and support new tissue development [16]. In addition, the high amount of fish collagen and glycerine improved the porosity, mechanical strength, biostability and cytocompatibility of the scaffolds [16].

In a similar study, better properties of chitosan-collagen (derived from tilapia fish skin) porous scaffolds were obtained by incorporating zinc oxide nanoparticles [17]. In this study, the 2.0% zinc oxide chitosan-collagen porous scaffolds were shown to have the highest fibroblast proliferation [17]. In a different study, electro spun PCL/ collagen (tilapia fish skin collagen) composite scaffolds with different contents of Nile tilapia skin collagen were fabricated and investigated for their biological activities [18]. The results indicated that L929 mouse fibroblasts were actively grown during the 5 days of cell culture without experiencing cytotoxic effects [18]. Due to the synergetic effects of PCL and collagen, the proliferation of L929 fibroblasts were found to be significantly higher on the PCL/collagen scaffold compared to that of control group [18]. The scaffolds with a collagen concentration of 8% and 10% were proved to be superior to others in cell adhesion and biocompatibility [18]. The fish skin collagen might affect intracellular signaling and cell response. In a different study, the hydrophilicity of Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) films was significantly increased by incorporating

tilapia fish collagen [19]. Subsequently, the collagen blend scaffold surfaces were found to have higher fibroblasts adhesion and growth than that of the control. In addition, improved cytocompatibility was reported in the collagen blend film [19]. Incorporating collagen with other polymer materials may result in better properties for skin tissue substitutes. The RGD peptide sequence found in collagen is recognized by the cell surface, allowing the attachment of cells to ECM.

Bone/dentin tissue engineering

Critical-sized defects in bone mainly caused by traumatic injury, bone-related diseases, primary tumor resection, or orthopedic surgery have in many cases may not be capable of repairing themselves by means of mechanical fixation alone. These defect scenarios need a substitutionary material to fill the bone defect. Several bone TE strategies, including acellular scaffolds, gene therapy, growth factor delivery, cell transplantation, and stem cell therapy, have been applied to address the above issues. Practically, bone TE requires the combination of the listed strategies. A tissue-engineered scaffold that mimics the complicated physiochemical attributes of bone may serve as a platform to incite the body's natural biological response to tissue damage and promote a natural healing process that does not occur in critical-sized defects. Various biomaterials, including ceramics, metals, polymers, and composites, have been studied for their potential as bone scaffold materials. Natural polymers especially collagen has been widely utilized in bone scaffold development due to its biological features, network and porous structures and mechano-elastic behavior suitable for bone TE purpose [20]. In addition, collagen fibers become the principal sources of tensile strength of bone tissues by providing a framework for hydroxyapatite deposition for further remodeling [21]. Collagen can be combined with other biomaterials such as hydroxyapatite, chitosan, calcium phosphate, and alginate to form scaffolds with different mechanical and biological properties [22]. Fish collagen becomes an emerging player for biomedical applications due to the pathological risk of mammalian collagen [23]. Furthermore, fish collagen peptides were proved to promote posttranscriptional modification for collagen maturation and gene expression for osteoblasts differentiation [24,25].

Unlike skin TE that utilized tilapia collagen mostly from the skin part, collagen derived from tilapia scales is commonly employed in bone TE applications. For instance, 3D porous scaffolds were fabricated by a combination of tilapia scale collagen and microbial transglutaminase (mTGase) enzyme to manipulate human mesenchymal stem cells to form osteogenic cells [26]. In this study, mTGase acted as a catalyst to preserve the inherent properties of collagen. The study was conducted by comparing the performance of tilapia scale collagen and porcine collagen on the biological properties of the fabricated scaffolds. The ALP activity of tilapia scale collagen-coated dish and scaffolds with or without mTGase were significantly higher than that of porcine collagen samples [26]. These results indicated that osteoblastic differentiation was greatly enhanced in the presence of tilapia scale collagen with/without mTGase. Furthermore, the late osteoblastic differentiation stage of hMSCs was shown 30-fold higher in the mTGase crosslinked tilapia scale collagen scaffolds than in the mTGase crosslinked porcine collagen scaffolds after being cultured for 3 weeks [26]. The early stage of osteoblastic differentiation in hMSCs was remarkably accelerated on a tilapia collagen surface due to specific fibril formation of tilapia collagen [27]. A fibrous collagen membrane was shown to have higher ALP activity than a non-fibrous collagen membrane even before adding osteoblastic differentiation medium, suggesting that the degree of the fibril formation of tilapia collagen affected the osteoblastic

differentiation of hMSCs. In addition, calcium deposition increased significantly in hMSCs cultured on tilapia collagen-coated dishes compared with porcine collagen-coated dishes, indicating tilapia collagen could facilitate the deposition process [27]. Effect of type I collagen derived from tilapia fish scale on odontoblast-like cells was also investigated [28]. Biocompatibility study of the collagen showed two-fold enhancement of the attached cells as compared to control. The cells were greatly induced to differentiate toward odontoblast lineage as proved by increased ALP activity on day 7, improvement of ALP, BSP mRNA expression on day 7 and 10, as well as enhanced mineralization on day 9 [28]. Biocompatibility of tilapia scale collagen was also evaluated for tissue regeneration in the oral-maxillofacial area [29]. Odontoblast proliferation, differentiation, and mineralization in tilapia scale collagen exhibited comparable performance to porcine collagen [29]. Since the future use of mammalian collagen may be hampered by religious restriction, bovine spongiform encephalopathy (BSE), foot and mouth disease, underutilized tilapia scale collagen offers a potential alternative for the mammalian collagen and might be useful for bone and dentin-pulp regeneration.

Neural tissue engineering

The nervous system is the most important system in the body since the sensory and motor functions are highly dependent on this system. Injuries to this system affect the body's functions and could be lethal for humans. However, due to the complexity of this system and its restricted ability to regenerate, the restoring process has always been a challenge for neurobiologists and neurologists. To date, several scientific approaches have been suggested to restore the function of a damaged nervous system, including cell therapies and TE [30]. Novel strategies that combined biomaterials, cells, and growth factors provide a potential solution to tackle these neurological disorders. Induced pluripotent stem cells (iPSCs) hold great potential for cell therapies and TE [31]. The ability of iPSC to differentiate and develop into functional cells is one of the crucial component in developing regenerative medicines [31]. A combination of biochemical factors and mechanical properties of the ECM could determine the fate of stem cells. Tissue stiffness of the ECM is one of the mechanical properties that affect the determination of iPSCs fate toward specific cellular subtypes [32]. For iPSCs to differentiate into neural lineage choice, the stiffness condition of living brain tissue must be reproduced *in vitro*. A study by Iwashita et al. has successfully mimicked the stiffness of living brain tissue *in vitro* using tilapia skin collagen gels [32]. The tilapia collagen gels were crosslinked with a combination of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxy succinimide (NHS) to produce a steady softer range of collagen gel that mimics brain tissue. A stiffness of 150-1500 Pa (like adult brain stiffness) with high reproducibility was obtained at a ratio of NHS to EDC is 0.1. Pluripotent cells were shown to differentiate into neural lineage and promoted the production of dorsal cortical neurons when exposed to the tilapia collagen gels [32]. These findings demonstrate that the tilapia collagen gel could be used for neural induction from pluripotent cells and provide a crucial development for neural regenerative applications.

Corneal tissue engineering

Corneal damage is a major cause of blindness worldwide, second only to cataracts. Trachoma, corneal opacities, and childhood blindness could lead to corneal blindness [33]. Currently, corneal transplantation is considered the main method for visual restoration treatment in corneal blindness patients. Full-thickness replacement of penetrating keratoplasty was the first

method used to perform corneal transplantation and prevails as the most common method [34]. Nevertheless, the availability of corneal donor tissue becomes the fundamental problem with the corneal replacement method. A severe shortage of donor tissue, limited access to drugs such as steroid and antibiotic, and lack of skilled surgeons, resulting in increased number of untreated patients [33]. Therefore, artificial corneal substitutes have been studied to overcome the shortage and problems associated with human donor corneas.

Synthetic prostheses and tissue-engineered constructs were developed to facilitate the regeneration of the host tissue and restore the cornea's refractive function [35–37]. 3D scaffolds made from biomaterials could mimic the corneal stroma and provide a suitable environment for the patient's own corneal cells to repopulate and regenerate [35,38,39]. The scaffolds can be synthetically fabricated or harvested in an almost ready state. The human corneal stroma comprises mainly of type I collagen, organized in orthogonal lamellae, resulting in enhanced tensile strength in the cornea [40]. Thus, collagen could be the most suitable material used to construct an artificial corneal scaffold. In addition, the already existing collagen scaffold in nature may reduce the fabrication cost and promise an adequate resource for clinical transplantation [41]. Fish scales are primarily composed of connective tissue protein and collagen (up to 81%), covered with calcium phosphate and calcium carbonate. Therefore, acellular and decalcified fish scales may serve as an effective collagen scaffold that induce regeneration of the damaged cornea by emulating the functions of the highly natural ECM scaffolding of the cornea.

Tilapia fish scales are known to have parallel-arranged collagen fibers, mimicking the human corneal stroma [42]. Several previous studies have reported the potential of decalcified tilapia fish scale collagen scaffolds for corneal regeneration [34,41–45]. Lin et al. developed decalcified tilapia scale collagen scaffolds to serve as an *in vitro* template for culturing the corneal cells [42]. The natural 3D microstructure sustains its initial structure even after being acellularized and decalcified. The fabricated scaffolds displayed good rabbit corneal cell proliferation and biosynthetic activity after 7 days of cultivation [42]. The micropatterned structures of the decalcified scales are not only facilitating cell attachment but also guiding cell migration through multiple parallel channels [42]. In a different study, the light-scatter and light-transmission properties of tilapia fish scale collagen matrix were investigated. The amount of scattered light was similar to that seen in an early cataract. Meanwhile, the light transmission was comparable to the transmission through the human cornea [41]. Rat keratoplasty model was used for corneal transplantation studies at three different surgical sites (anterior lamellar keratoplasty-ALK, interlamellar corneal pocket-IL, and subconjunctival-SC). Different degrees of haziness, pupil obscureness, and inflammation were generally seen at those implanted sites [41]. This experiment showed that the fabricated decellularized scaffold has sufficient light transmission values and is suitable for use in keratoplasty. The same research group performed an in-depth study to determine the suitability of a tilapia fish scale-derived collagen matrix for corneal reconstruction [45]. The results showed no cytotoxicity effects, normal phenotype markers, and no inflammation or sensitization. Moreover, the implanted corneal led to a transparent cornea, healthy epithelium, and no immunogenic response [45].

In a separate experiment, morphological and physiological properties of decalcified tilapia scale collagen implants were studied using 6 months of follow-up of rabbit model [34]. The implanted

cornea displayed a clear surface with no haze and ulcer detected up to 6 months postoperatively. In addition, no immune response, dissolution, fragmentation, and degeneration were observed after a long-term evaluation [34]. The potential of acellular and decalcified tilapia fish scale collagen as an ideal artificial cornea substitute was also proved by investigating its biocompatibility towards primary human corneal endothelial cells (HCEnCs) [44]. In line with the previous research, the scaffolds displayed correct morphology, cytocompatibility, and no toxicity for HCEnCs [44]. Previous studies revealed that the new approach of using acellular and decalcified tilapia fish scale collagen scaffold might yield an ideal artificial cornea substitute for long-term inlay placement. However, regulatory compliances like that of advanced therapy medicinal products are needed for further clinical use.

Conclusions

The review clearly narrates that tilapia fish collagen has potential uses in TE. Considering the factors involved in scaffold fabrication, such as denaturation temperature and issues related to biological safety, collagen originating from tilapia fish is thought to be a suitable biomaterial to replace mammalian collagen for use in clinical regenerative medicine. The higher thermal stability of tilapia fish collagens compared to other marine sources justifies its utilization in TE. However, further animal experiments are needed before the collagen can be applied clinically.

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