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FISH GELATION IN SKIN AND BONE TISSUE ENGINEERING

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ABSTRACT

Gelatin is a biomaterial commonly used in tissue engineering application as it offers biocompatibility feature that can attract cell attachment. The most abundant sources of gelatin usually come from porcine skins, bovine hides and cattle bones. However, the mammalian gelatins are rejected by some consumers due to religious reason or health concerns. The fish gelatin can become a good option to substitute the mammalian gelatins. This review provides insights on the use of fish gelatin in tissue engineering applications. The review is focusing on the application fish gelatin in two main areas which are skin tissue engineering and bone tissue engineering that been studied by researchers in journals and thesis report. The potential of fish gelatin to substitute mammalian in this field will provide an alternative for Muslims to choose a halal medical treatment for them.

Key words: Fish gelatin, gelatin, halal, tissue engineering

INTRODUCTION

Gelatin is one of the natural materials used in tissue engineering applications. The scaffolds or grafts have been intensively used for tissue engineering due to their ability to support cell adhesion and proliferation. Gelatin found from porcine skin makes about 50% of the world manufacturing and is largely consist of collagen removed from the skin through acidic baths or alkaline and thermal procedures. Collagen of animals such as chickens, cattle, porcine and fish are converted into a polymer called gelatin. Collagen is mostly found in tendons, ligaments, bones and cartilage ('Public Health England, 2015). The suspension rates of gelatin are very low due to its ability to create cross-links structure in the denatured collagen chains under certain environments which can neutralize the gel system.

Gelatin might comprise particles other than collagen such as sugars, lipids and other proteins which can interact with collagen network in order to create covalent bonds due to it been extracted from animal tissues. The most profuse sources for gelatin production are from porcine skin (46%), bovine hides (29.4%) and porcine and cattle bones (23.1%) while it been reported that fish gelatin produces less than 1.5% of total gelatin production in 2007 (Duconseille *et al*., 2015).

The porcine and bovine gelatins are used intensively in various types of tissue engineering due to their stability and morphological properties. But, these mammalian gelatins have a problem regarding their halal status which makes Muslim consumers concerns about their usage effects. In order to counter this problem, fish gelatin provides an alternative to substitute mammalian gelatins in tissue engineering applications. However, there are fewer studies have been done on the use of fish gelatin in tissue engineering. Therefore, this paper aims to review the application of fish gelatin in tissue engineering.

Tissue engineering

Tissue engineering (TE) suggests a possible solution that introduces novel methods for tissue repair and overcomes the deficiency of transplantable organs. TE mainly consists of several

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components which are signaling molecules, flexible scaffold and cell source, that can improve the cell biology from its proliferation ability to special features of cell differentiation.

The purpose of tissue engineering is to develop complex living constructs. The functional substitutes can be implanted to enable cell attachment, proliferation and differentiation by providing biological microenvironment through Extra Cellular Matrix (ECM) synthesis (Pandey *et al*., 2017). Nanofiber structures have been marked for the preparation of scaffolds for tissue engineering. Biocompatible and biodegradable fibrous scaffolds are ideal over conventional scaffolds for the regeneration of tissue. This is because of their unique nature and capability to provide the target cells or tissues with a natural environment by imitating the extracellular matrix. There are many biomaterials that have been used in tissue engineering such as collagen, gelatin, chitosan, polycaprolactone and silk (Selimoviæ *et al*., 2015).

TE approach had brightly improved in these recent years, but it still has room for improvement. Many problems need to be tackled in order to use engineered tissues and organs such as bio-compatibility, immunogenicity, biodegradation and toxicity of the products (Edgar *et al*., 2016). The nature of biomaterials used as scaffolds in TE also needs to be tested to enhance TE applications. Appropriate biomaterials must have the ability to imitate the properties of desired tissues, their functional roles and their extracellular matrix which can increase the success rate of TE (Edgar *et al*., 2016).

TE allows the study of human physiology *in vitro* through the engineered tissue substitutes (Castells-sala *et al*., 2015). The 3D Extracellular Matrix (ECM) ensures the growth of cells in the body while surrounded by other cells in order to have organized structures. In fact, the cell-cell and cell-ECM connections can regulate whether a specified cell experiences proliferation, differentiation, apoptosis or invasion conditions. The three basic elements which are scaolds, cells and biomolecules will make up the TE system (O'Brien, 2011).

The gelatins have been a popular usage in tissue regeneration and regenerative medicine areas including skin tissue engineering, vascular tissue engineering, cartilage tissue engineering, bone tissue engineering, drug delivery system and wound dressings (Chong *et al*., 2007; Castells-sala *et al*., 2015; Benjakul & Oungbho, 2017). This review will be focusing on the use of fish gelatin in skin tissue engineering and bone tissue engineering.

Fish gelatin

Fish gelatin is extracted from aquatic sources including warm- and cold-water fish skins, bones, and fins and can become a potential substitute for porcine and bovine gelatins (Duconseille *et al*., 2015; Edgar *et al*., 2016). The utmost benefit of fish gelatin is that they will not be associated with the danger by epidemics of Bovine Spongiform Encephalopathy (BSE) and can comply with halal requirements. The use of fish gelatin is acceptable for Islam and can be used with minimal restriction in Judaism and Hinduism. Moreover, gelatin can be extracted from fish skin, the major by-product of the fish-processing industry (Badii & Howell, 2006).

The abundant contents of collagen can be found in fish skin components. According to Nagai & Suzuki (2000), the collagen contents for fish skin waste of three species of fish which are Japanese sea-bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively. The mammalian gelatins (porcine and bovine) have higher rheological properties than fish gelatin (Gomes *et al*., 2015; Gomes *et al*., 2017). The important aspects which influenced the gelatin characteristics are the nature of original collagen and their extraction methods (Gomes *et al*., 2015; Gomes *et al*., 2017). In addition, various fish types differ significantly in the amino acid composition of collagen (Alfaro *et al*., 2014). Nevertheless, the abstraction procedure is very crucial because it can control the molecular weight of gelatin at the end of the process (Gómez-Guillén *et al*., 2009).

However, fish gelatin possesses lower gelation and melting temperatures and exhibits lower gel strength compared to mammals gelatin (Alfaro *et al*., 2014). The crucial property of gelatin is the gel strength which is vital in order to determine the success of the end product (Alfaro *et al*., 2014). Moreover, the gel strength or Bloom also acts as a functional property of gelatin as it mostly associated with the battle of gelatin towards degradation process (Alfaro *et al*., 2014). The influence aspects of fish gelatin which affected the gel strength including molar mass, concentration of gelatin solution, the temperature and aging time of the gel and pH while, the range of Bloom value of fish gelatin usually ranged from zero to 270 g (Karim & Bhat, 2009). The cold-water fish species display gel strength values of 100 g or lesser, meanwhile, the warm-water fish gelatin has more than 200 g for gel strength values (Gomez-Guillen *et al*., 2011). The viscosity property of gelatin become the second most important property after gelatin gel strength (Alfaro *et al*., 2014). The gelatin with low molecular weight will reduce the gel strength and viscosity

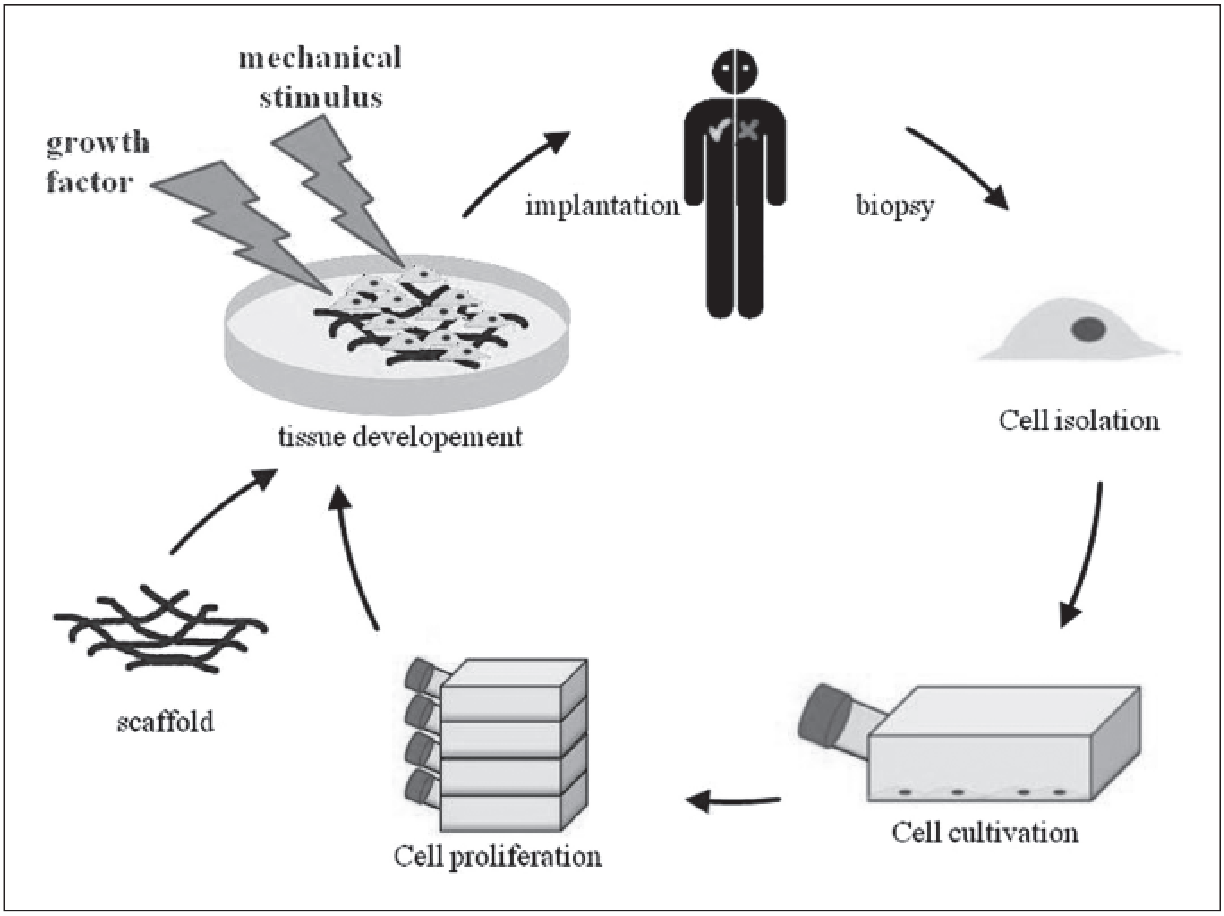


Fig. 1. Introduction to tissue engineering.
Source: Castells – Sala (2015)

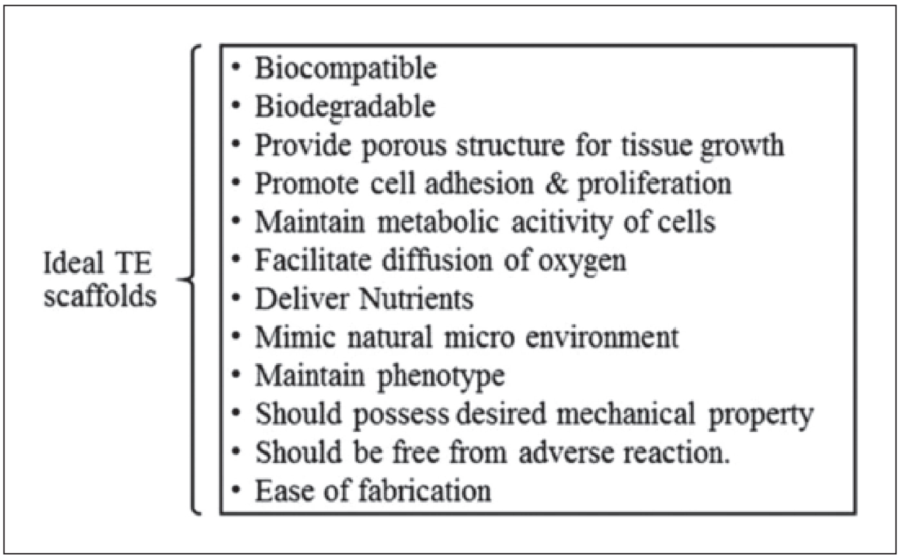
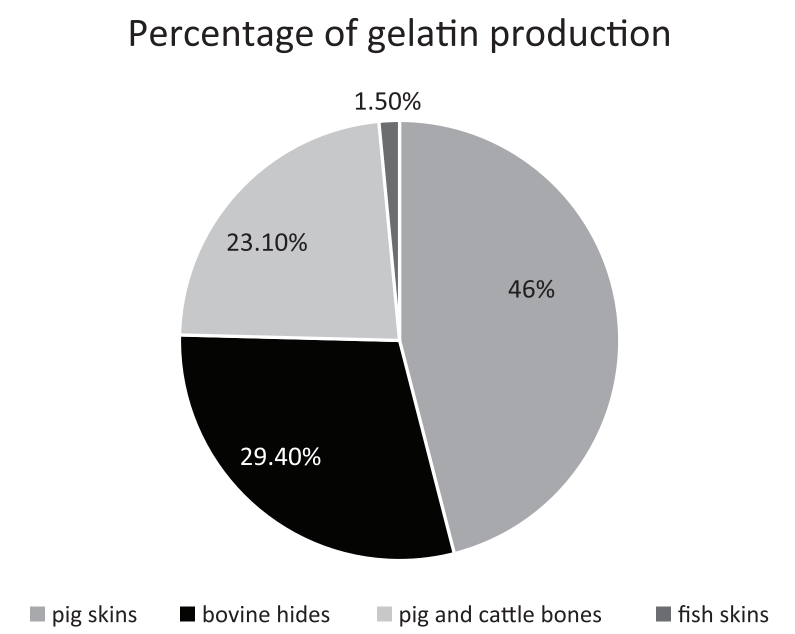


Fig. 2. Characteristics of scaffolds in tissue engineering.
Source: Pandey *et al.* (2017)



Source: Duconseille *et al*. (2015)

Fig. 3. World gelatin production.

of the solution (Alfaro *et al*., 2014). The factors which affected the differences of viscosity among fish gelatins are mostly due to their extraction environment, the variety of fish types and gelatin molecular mass (Alfaro *et al*., 2014).

The rheological properties of fish gelatin can be improved by crosslinking process with polymer agents which have been studied in most of the studies regarding gelatins. In fact, the gelatin altering ingredients such as salts, glycerol, glutaraldehyde, enzyme, sugars and others (Duconseille *et al*., 2015), have a role in preparing the required characteristics. Other than that, the fish gelatin can enhance their lower rheological properties by using protein-polysaccharide crosslinking interactions (Duconseille *et al*., 2015). These days, the crosslinking connections have been attracted many researchers which have been practiced in tissue engineering applications (Duconseille *et al*., 2015).

Fish gelatin in skin tissue engineering

The demand for possible skin grafts has encouraged researchers to find suitable substitute through skin engineered approach which can accelerate the healing process of defected skin areas. The period of skin tissue engineering was indicated a long time ago with a bi-layered and the bovine collagen scaffold able to encourage the mixture of a neo-dermis (Yildirimer *et al.,* 2012). This original bio-construct (Integra) revolutionized the existing skin injury treatments and always used as a benchmark in severe full-thickness burns (Yildirimer *et al.,* 2012). There are numerous choices and suitable alternatives for skin engineered constructs and they are being extensively studied and some of them have been commercialized (Yildirimer *et al*., 2012).

Gelatin is one of the biomaterials used to prepare tissue-engineered skin as it offers biocompatibility and bioactivity for cell attachment purpose. The presence of RGD (Arginine-Glycine-Aspartic) amino acid sequence in gelatin molecular structure provides an essential binding site for cell interactions that mimic the natural extracellular matrix. Mammalian gelatin is often been utilized for the preparation of nanoscale gelatin fibres. However, the electrospun mammalian gelatin fibres are constrained in Islamic law if it is derived from porcine and bovine that is not slaughtered properly. Therefore, fish gelatin is a better alternative for the engineering of skin tissue.

In one of the studies, the cold-water fish skin gelatin has been fabricated while being compared with scaffolds from porcine and bovine for skin substitutes (Kwak *et al*., 2017). The fabrication of fish skin gelatin scaffold was using the electrospinning method and crosslinked with glutaraldehyde vapor (GTA) (Kwak *et al*., 2017). The fish gelatin has lower contents of proline (Pro) and hydroxyproline (Hyp) compared to mammalian gelatins (Gómez-Guillén *et al*., 2009). Fish gelatin also retains a sol condition without display any gelation behaviour at room temperature (Gómez-Guillén *et al*., 2009). The crosslinking process happens due to the hydrophilic properties of gelatin. This crosslinking process is one of the most practical methods which can enhance the mechanical characteristics of the nanofiber scaffolds (Wang *et al*., 2016). The result of cell viability analysis proven that the nanofiber fish gelatin scaffolds have good cell adhesion and high proliferation rate due to the uniform diameter of pore structure, similar to that of ECM (Kwak *et al*., 2017). The hydrophilic surface of fish gelatin assists the attachment of fibroblast cells to scaffold easily (Ranella *et al*., 2010).

In a different study, the fabrication of skin engineered implant was made through the combination of cold-water fish gelatin, poly-caprolactone (PCL) and chitosan by using electrospinning method (Gomes *et al*., 2015). The fish gelatin was crosslinked with glutaraldehyde vapor (GTA) and been compared with PCL and chitosan scaffolds in terms of cell-scaffold interaction and wound healing promotion (Gomes *et al*., 2015). From the result of porosity, the values for three scaffolds are within range of 70 to 90% which can be considered as suitable to be used as skin substitute (Chong *et al*., 2007). The cell viability results showed that the chitosan scaffold having least cell attached on the scaffold compared with PCL and gelatin-GTA scaffolds due to the absence of RGD sequences that promotes cellular interactions (Fang *et al*., 2005). The gelatin-GTA scaffold reached the highest number of cells *in vitro* compared to PCL and chitosan scaffolds (Gomes *et al*., 2015). Accordingly, the combination of gelatin with other polymers such as PCL and chitosan will increase its stability and enhance the wound healing process in order to become a good candidate of skin substitute for skin tissue engineering.

The fabrication of fish skin gelatin with GTA was also been studied for dermal replacement in the wound healing process (Shevchenko *et al*., 2014). The gelatin-GTA scaffold has small pore structures compared to Integra skin substitute which can limit the cells that can be attached to the framework. However, the attachment of cells to the pore walls can initiate proliferation in the extracellular matrix (ECM) (Ghosh *et al*., 2007). The degraded mechanical property of the gelatin and GA scaffold is possibly correlated to its reduced preliminary thickness and the original phases of scaffold biomodification (Shevchenko *et al*., 2014). This remark showed that the combined gelatin and GTA scaffold might be altered faster and degraded in the *in vivo* atmosphere compared to the other scaffolds. Thus, it is become problematic to simplify *in vivo* biodegradation rates from *in vitro* data as the wound atmosphere is complicated, with an array of prosynthetic factors, such as cellular and humoral components (e.g. fibroblasts and TIMP cytokines), as well as cellular and humoral factors intended at matrix biodegradation (macrophages and matrix degradation enzymes) (Kondo & Ishida, 2010).

In one of the studies, an attempt has been made to compare the performance of scaffolds derived from cold water fish gelatin-chitosan-PCL polymers using electrospinning (Gomes *et al*., 2017). Four samples were fabricated; chitosan-PCL, chitosan-gelatin, PCL-gelatin and PCL-gelatin-chitosan. It was revealed that PCL-gelatin-chitosan scaffolds have good result in term of physical properties while PCL-gelatin scaffolds can enhance cell adhesion ability (Gomes *et al*., 2017).

Fish gelatin in bone tissue engineering

In order to restore the function of damaged and degenerated bone, tissue engineering approach offers a very promising alternative. By combining dramatic advances in biomaterials, biological evaluation and engineering analysis, bone tissue engineering technique creates unprecedented choices for synthetic scaffold selections (Zhang *et al*., 2016). Numerous types of synthetic scaffolds have been fabricated and investigated in finding effective bone graft substitutes (Wu *et al*., 2015). Gelatin is one of the common biomaterials used in constructing bone graft scaffold as it offers biocompatibility and bioactivity (Xia *et al*., 2012). Gelatin mostly used as a coating agent, nanofibrous composites, injectable gel or bone filling material (Linh & Lee, 2012). Most of the gelatins used in this field were derived from mammalian sources. However, as the awareness of choosing Halal products has risen among Muslim worldwide, the concern of providing Halal medical treatment become more visible. Therefore, some of the researchers took an initiative to replace mammalian gelatin with fish gelatin for bone tissue engineering purpose.

Previously, the fish skin gelatin from unicorn leatherjackets (*Aluterus Monoceros*) was fabricated and incorporated with chitosan for application in bone tissue engineering (Benjakul & Oungbho, 2017). The fabrication of fish gelatin and chitosan scaffolds had to be crosslinked with glutaraldehyde by using the freeze-drying method because of the lower gel strength of fish gelatin (Benjakul & Oungbho, 2017). The scaffolds were nontoxic to cells, supported biomineralization and promoted cell attachment (Benjakul & Oungbho, 2017). The surface characteristics of the scaffolds play an important role in tissue engineering as they affect hydrophilicity, nutrient supply, cell ingrowth and proliferation. If the pore size is too small, the seeded cells can attach only onto the outer surface of the scaffolds and the migration of cell into the inner parts of the scaffold is impaired (Miyazaki *et al*., 2009). The gelatin and chitosan scaffolds revealed the suitable pore size to accommodate the cell attachment.

In a different study, deep-sea fish skin gelatin was crosslinked with glutaraldehyde for bone tissue engineering applications by using electrospinning technique (Zhan *et al*., 2016). The results revealed that the gelatin- glutaraldehyde scaffold has a nice uniform diameter and more than 80% of porosity (Zhan *et al*., 2016). In fact, porosity is an important factor which can evaluate the quality of scaffold for tissue engineering applications (Zhan *et al*., 2016). The crosslinked gelatin can form a 3D network structure and the molecular chain is restrained by the crosslinking point that can endure large external force (Zhan *et al*., 2016). These factors will make the scaffolds have strong mechanical property.

The study on the fabrication of bone grafts by using cold water fish gelatin has been produced in this research (Yoon *et al*., 2016). The cold- water fish has been used and converted into gelatin Methacryloyl (GelMA) hydrogels for tissue engineering (Yoon *et al*., 2016). The result showed that bigger scale of GelMA synthesis process has been produced and the fabrication of hydrogel can occur at room temperature due to the low melting values of fish gelatin compared to porcine gelatin (Yoon *et al*., 2016). The result of mechanical strength displayed that the fish gelatin has a low mechanical property due to they have lesser amount of hydrophobic amino acids (alanine, valine, leucine, isoleucine, proline, phenylalanine and methionine) and imino acids (proline and hydroxyproline) compared to mammalian gelatin (Badii & Howell, 2006),(Araghi *et al*., 2015*)*. Therefore, gels from fish GelMA will not having enough hydrophobic interaction and can simply be destructed even by the small force. The imino acids which are the source to the structural stability of the hydrogels only present in small amount in fish gelatin and make the hydrogel less stable. But, the fish gelatin GelMA can be crosslinked in order to make it become more stronger and stable (Muyonga *et al*., 2004). In conclusion, the fish Gel MA hydrogel can be the promising candidate to be used in tissue engineering applications.

CONCLUSION

In recent years, there are tremendous concerns about the sources of the gelatin used in tissue engineering applications. The needs to find the alternative for the mammalian gelatins become rapid day by day. The replacement towards porcine and bovine gelatins happen due to safety and halal requirements. Fish gelatin can be considered a good choice for substitution. The using fish gelatin to substitute the traditional gelatins in the market can create other option for the population who does not consume the mammal-derived products. The by-product of fish in the fishing industry can become one of the alternative sources for the production of fish gelatin used for tissue engineering and other applications. Moreover, this process will ensure the safe process, low in cost and can avoid the environmental pollution caused by fish by-product wastes. In the future, the fish gelatin can become a practical substitute biomaterial in industry and tissue engineering fields.

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