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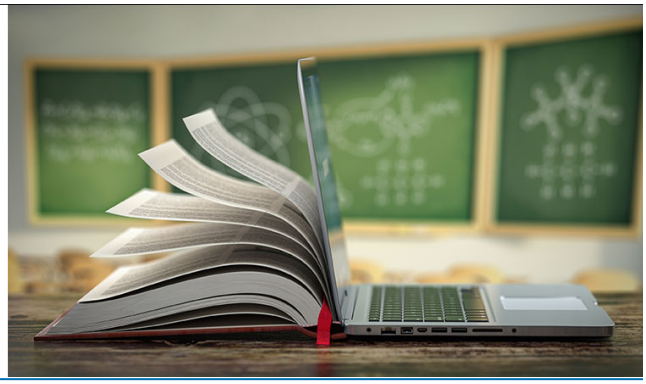

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Properties of Tilapia Collagen as a Biomaterial for Tissue Engineering: A Review

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Abstract. Collagen is one of the common biopolymers used as a biomaterial in tissue engineering applications due to its biocompatibility and biodegradability properties. Collagen derived from various sources and mostly found in porcine and bovine skin. However, due to religious concerns and the dangers of animal-borne diseases possesses by these mammalian derived collagens, marine collagens are extensively investigated as an alternative to substitute mammalian collagens in tissue engineering applications. Among those marine collagens, tilapia (*Oreochromis niloticus*) collagens are known to have a great potential to be used as biomaterials for tissue engineering application due to its higher thermal stability compared to other marine sources. Therefore, this study aims to review chemical and biological properties of tilapia collagen as a biomaterial for tissue engineering applications. The contents are mainly focused on the extraction yield, amino acid composition, thermal stability, cross-linking, biocompatibility, biodegradability, immunogenicity and hemostasis of tilapia collagen.

1. Introduction

Collagen is a multifactorial biomaterial that is widely used in the advanced biomedical technologies including tissue engineering (TE) technology.

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Commonly, collagen is extracted and purified from the mammalian body such as porcine and bovine. However, due to religious constrain and a great pathological risk for transmitted diseases including bovine spongiform encephalopathy, avian and swine influenza, and foot-and mouth disease in bovines, pigs, and buffalo, which frequently occur worldwide, the use of these mammalian collagen for TE

purpose should be replaced by other safer alternatives [1]. Collagen originating from marine sources were proved to have a great potential in substituting mammalian collagen.

Among those marine sources, tilapia collagen was found to be an ideal candidate to replace mammalian collagen due to its higher thermal stability compared to other marine sources [2]. In addition, tilapia is one of the main groups of fish grown and sold as whole frozen fish and fresh fillet [2]. Therefore, during the industrial processing, approximately 60 - 70% byproducts are fabricated including skin, scales and bones [3]. These parts are rich in collagens and other bioactive molecules and mostly, collagens are extracted from Nile tilapia (*Oreochromis niloticus)* species to be used as biomaterials [2].

Tilapia collagen has long been used as one of the natural biomaterials for biomedical applications especially in TE. The unique properties of tilapia collagens have make them a very promising candidate to substitute mammalian collagens. Thus, this study aims to review chemical and biological properties of tilapia collagen as a biomaterial for TE applications. The contents are mainly focused on the extraction yield, amino acid composition, thermal stability, cross-linking, biocompatibility, biodegradability, immunogenicity and hemostasis of tilapia collagen.

2. Chemical Properties

2.1. Yield of extracted collagen

Acetic acid and pepsin hydrolysis methods are the common methods used to extract collagens from skin, scales and bones of tilapia. The yield of collagen is expressed by a basis of lyophilized dry weight and can be calculated from the following equation:

$$Yield\ (\%) = (Weight\ of\ lyophilized\ collagen)\ (Weight\ of\ initial\ dry\ fish\ byproduct)^{-1} \times 100$$

Collagens extracted from tilapia skin are shown to have higher yield compared to other byproducts. A study by Chen et al.[4] has reported the higher collagen yield was obtained from tilapia skin which is 27.20% when compared to tilapia scales with 3.20% collagen yield which has been isolated through an acetic acid method. However, Huang et al.[5] has reported that greater collagen yield of 16.60% was obtained from tilapia scales when the scales were extruded rather than using the conventional acetic acid method.

Table 1 lists the yield and denaturation temperature of collagen extracted from various tilapia byproducts using different extraction methods. From Table 1, the yield of collagen from tilapia byproducts is greater when the extraction was performed using a pepsin hydrolysis method when compared to acetic acid method. For instance, the yield of collagen from tilapia skin was increased by 0.23% when pepsin was used during the extraction process [6]. A study by Potaros et al.[7] also proved that higher collagen yield was obtained from tilapia skin through a pepsin hydrolysis method either using an approach by Noitup et al.[8] or Ogawa et al.[9]. The increment of collagen yield is likely caused by cleaving of cross-linked molecules in the telopeptide region during the pepsin digestion process, resulting in the increment of collagen extraction efficacy [10]. The telopeptides play an important role in providing a covalent aldol cross-linking of the triple-helix structure. Excision of the telopeptides leads to an incomplete collagen protein structure and increases collagen solubility, therefore contributes to a higher extraction of collagen yield [11]. However, the removal of telopeptides by pepsin will produce unstable thermal structural that can lead to lower denaturation temperature of pepsin soluble collagen (PSC) [6]. Moreover, from the view of biomedical materials, the telopeptides will affect the immunogenicity of

collagen [12]. The peptides which are located at the center of triple helix of pepsin-treated skin collagen will be the major antigenic site to activate human immune response [13]. While, another extraction method using an acetic acid has produced a lower collagen yield which might be due to low solubility cross-links that are formed through the reaction of aldehyde with lysine and hydroxylysine at the telopeptide helical sites [14].

Table 1: The yield and denaturation temperature of collagen extracted from various tilapia fish byproduct using different extraction methods.

Tilapia byproduct	**Extraction method**	**Yield (basis of lyophilized dry weight) (%)**	**Proline and hydroxyproline amino acid percentage (%)**	**Denaturation temperature, T_d (°C)**	**Reference**
Skin	Acetic acid method	19.80	20.03	34.5	[6]
	Pepsin hydrolysis method	20.03	19.15	30.0	
	Acetic acid method	27.20	19.70	-	[4]
	Acetic acid method	-	18.90	35.2	[15]
	Pepsin hydrolysis method	-	20.50	34.5	
	Acetic acid method	38.84 (Noitup method) 20.70 (Ogawa method)	19.40 (Noitup method) 19.80 (Ogawa method)	34.3 (Noitup method) 34.4 (Ogawa method)	[7]
	Pepsin hydrolysis method	48.21 (Noitup method) 38.27 (Ogawa method)	19.70 (Noitup method) 19.80 (Ogawa method)	34.3 (Noitup method) 34.6 (Ogawa method)	
	Acetic acid method	39.40	21.00	32.0	[16]
	Acetic acid method	25.60	-	32.5	[17]
	Acetic acid method	-	19.20	36.1	[12]
	Pepsin hydrolysis method	-	18.50	34.4	
Scale	Pepsin hydrolysis method	-	24.50	32.1	[18]
	Acetic acid method	3.20	20.70	-	[4]
	Extrusion–hydro-extraction (EHE) process	16.60	17.00-17.50	-	[5]
Bone	Acetic acid method	2.50	16.00	-	[19]
	Pepsin hydrolysis method	7.30	15.60	32.5	
Skin, scale and fin	Acetic acid method	22.00	-	32.0	[20]
	Pepsin hydrolysis method	56.00	-	29.0	

2.2 Amino acid composition

The composition of amino acid in collagens is usually expressed as amino acid residues per 1000 total amino acid residues. In record, aquatic origin collagens have shown similar amino acid composition to mammalian collagens with a major amino acid, approximately 1/3 of total residues [1]. The repetitions of proline-rich tripeptides (Gly-X-Y), where X is mostly proline and Y is mostly hydroxyproline, are crucial for the formation of collagen triple helix structure [21]. The high content of hydroxyproline

indicates high thermal stability of extracted collagens. This might be due to the role of hydroxyproline in the formation of intramolecular hydrogen bonds [22]. In addition, the hydroxylation of proline residues also plays a critical step for the helix stability. Yamada et al. has clarified that the hydroxylation degree of proline in fish collagens has reached 35 - 48%, similar to mammalian collagens [1].

Collagen extraction methods also have significant impact on the composition of amino acid . In a study by Sun et al., the proline hydroxylation rate of tilapia skin was found to be 41.8% and 42.0% using acetic acid method and pepsin hydrolysis method, respectively [15]. In addition, the composition of amino acid in collagens might be different depends on the types of tilapia byproducts. For instance, collagens derived from tilapia skin were reported to have higher amounts of alanine, glycine and glutamine but lower amounts of valine, hydroxyproline, proline and aspartic acid compared to collagens derived from tilapia scales [4].

2.3 Thermal stability

In general, the denaturation temperature of collagens, isolated from marine fish scales are between 26°C and 29°C, and between 28°C and 30°C for marine fish skin. These ranges of temperature are close to ambient temperature which limiting the manufacturing process of fish collagens [23]. Besides, this denaturation temperature can drastically alters the physiochemical, biological and mechanical properties of collagen [24]. Based on Table 1, the denaturation temperature of tilapia byproducts are found to be higher than marine collagens, which imply an advantage for its application in the manufacturing biomedical materials [12]. A slight difference is observed between the denaturation temperature of tilapia collagens derived using acetic acid method and pepsin hydrolysis method for both skin and scales [6,12,15,20]. Apparently, the loss of telopeptides by pepsin has affected the stability of triple helix structure, resulting in lower thermal stability, compared to the denaturation temperature of collagens, isolated using acetic acid method [6,12]. Nevertheless, this shortcoming can be surpassed by chemical cross-linking using various cross-linking agents.

2.3 Cross-linking for stability

Previously, tilapia skin collagen sponges, fabricated using a freeze-drying method, were physically cross-linked using dehydrothermal treatment and chemically cross-linked with glutaraldehyde to improve mechanical, biological and biodegradation behaviors [25]. The glutaraldehyde cross-linked collagen sponges were found to offer a higher cross-linking degree, superior mechanical properties and better liquid absorption in comparison with the dehydrothermal cross-linked collagen sponges [25]. A hygroscopicity assay verified that the stability of collagen in solution was improved after both cross-linking treatments where greater hygroscopicity was found on the glutaraldehyde cross-linked collagen sponges compared to the dehydrothermal cross-linked collagen sponges [25]. Hygroscopicity is the capacity of a material to react with moisture content of air by absorbing or releasing water vapor. It is an important parameter to determine the interaction of biomaterials with physiological fluid *in vivo* that significantly affects cell adhesion, growth, behaviour and differentiation.

In a different study, tilapia scales collagen was enzymatically cross-linked with microbial transglutaminase to enhance the mechanical strength of a 3D porous collagen scaffold [26]. The spectroscopic observation indicated that no deformation of collagen walls or blocked pores was detected after the cross-linking treatment [26]. In addition, enhanced expression of osteoblastic differentiation was observed on the cross-linked collagen scaffolds, making them a viable material for bone TE [26]. The performances of different cross-linking agents (N-hydroxysuccinimide (EDC/NHS); genipin and PBS; genipin and ethanol; tea polyphenol (TP); nordihydroguaiaretic acid (NDGA); and diphenylphosphoryl azide (DPPA)) on the physico-chemical properties, *in vitro* blood clotting and microstructure of tilapia skin collagen were evaluated by Sun et al.[27]. Among the listed cross-linking agents, collagen sponge cross-linked with EDC/NHS was proven to demonstrate highest hygroscopicity and better porosity [27].

Collagens isolated from different fish species have also been cross-linked to improve stability and other properties. For instance, collagens isolated from silver carp (*Hypophthalmichthys molitrix*) were chemically cross-linked with 2,3-dialdehyde cellulose (DAC) [28]. The stability of the treated collagens was greatly enhanced, indicated by the increase of denaturation temperature (T_d) of DAC cross-linked collagen [28]. In another study, the utilization of N-hydroxysuccinimide activated adipic acid (NHS-AA) as a cross-linking agent is also intended to improve the stability of collagen isolated from skin of large fin long barbel catfish (*Mystus macropterus*) [29]. Therefore, in general, these cross-linking treatments could provide a feasible method to improve stability, mechanical and biological behaviours of collagens isolated from marine sources, thus increasing the adoption of mammalian collagens to be used as biomaterials.

3. Biological Properties

The use of fish collagen as a biomaterial has been widely recognized due to its excellence biocompatibility, high degree of biodegradability, low immunogenicity and high coagulation capability [1]. Various biomedical applications especially in the field of TE have integrated fish collagen features into the fabrication of prosthetic devices in the form of three dimensional (3D) scaffolds, wound dressings, fibrous matrices and hydrogels.

3.1 Biocompatibility

Recent studies are focusing on the potential of warm water fish collagen especially tilapia, in substituting mammalian collagens for medical applications [2]. In one of the studies, evaluation on the biocompatibility of tilapia skin collagen sponges were investigated using an acute systemic toxicity assay where a porcine collagen was selected as the comparison group [12]. The results showed no significant differences in body weight of the tested mouse between the porcine collagen tested group and the tilapia skin collagen tested group after the injection of leach liquor, implying the collagen sponges are freed from acute systemic toxicity [12]. In addition, tilapia collagen treated group exhibited higher cell proliferation rate than the porcine collagen treated group in all MC3T3E1, L929 and HUVEC cells [12].

Active proliferation of baby hamster kidney (BHK-21) fibroblast cells, with no sign of toxicity was also observed in a gel containing 0.5% and 0.3% (w/v) of tilapia scales collagen [18]. The ability of tilapia collagen to facilitate higher cell proliferation rate might be due to the presence of an antibacterial tilapia piscidin (tp4) which can stimulate cell proliferation and activate epidermal growth factor (EGF), transforming growth factor (TGF) and vascular endothelial growth factor (VEGF) [30]. In another study, porous scaffolds developed from a blend of chitosan, tilapia scales collagen and glycerine have excellently facilitated human fibroblasts and keratinocytes cells adhesion and spreading [31]. Furthermore, the proliferation rate of human fibroblasts was increased as the concentration of tilapia collagen and glycerine increased, but remained stagnant for the human keratinocytes [31]. While, the efficiency of tilapia scales collagen in promoting odontoblast adhesion, differentiation and proliferation was addressed by Tang et al.[32] where a rat odontoblast-like cell line (MDPC-23) was proved to show favourable growth on a collagen treated sample due to interaction between the collagen and the cell surface integrin, which in turn activated a series of intracellular signal pathways [32].

3.2 Biodegradability

Collagen is classified as an enzymatically degradable natural polymer. It undergoes enzymatic degradation within the body, producing corresponding amino acids via the action of enzymes such as collagenases and metalloproteinases. The enzymatic degradability of collagen leads to its unique physio-chemical, biological and mechanical properties, which has been widely investigated for biomedical applications. The susceptibility of collagen towards biodegradation is expressed as a hydrolysis percentage of collagen exposure to enzymes (eg. bacterial collagenases and metalloproteinases), and analyzed by the content of hydroxyproline [25]. Previous studies have shown

that the alteration of collagen degradation rate is possible with the aid of enzymatic pre-treatment or cross-linking using various cross-linking agents. *In vitro* degradation studies have revealed a higher stability of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) cross-linked scaffolds derived from tropical fresh water fish scales collagen, with only ~50% reduction in mass after 30 days, whereas the uncross-linked scaffolds were completely degraded within four days [33].

In different study, tilapia skin collagen sponges were chemically cross-linked with various cross-linking agents and its biodegradation against collagenase was studied [25,27]. The results revealed that the biodegradation rate of all cross-linked collagen sponges were reduced to different extents, implying higher susceptibility toward collagenase digestion [25,27]. As an example, the biodegradation rate of glutaraldehyde cross-linked tilapia skin collagen has been significantly reduced to 46.20% [25]. Collagenase can preferentially cleaves a unique site of X-Gly bond in a specific sequence of leucine and glycine in collagens [34]. The resistance is mainly due to the masking of collagenase active site which resulted from the cross-linking [35,36]. Besides, the *in vitro* and *in vivo* degradation properties of snakehead scales collagen patches were also investigated [37]. The presence of collagenase significantly enhanced the degradability of uncross-linked collagen patches which has been proved by the degradation of 30 wt% as compared to 7wt% by the end of degradation treatment [37]. Meanwhile, an *in vivo* study assessed by Masson's trichrome staining revealed that the uncross-linked collagen patches were almost completely degraded after 21 days of implantation as compared to 5 wt% cross-linked collagen patches [37].

3.3 Immunogenicity

Even though collagens have been proved to have low toxicity and low probability to induce an immune response [2], a study by Bentkover[38] has found that bovine collagens possessed some immunogenicity, whereas porcine collagens might trigger allergic reactions and IgG elevation. Therefore, the immunogenicity of tilapia collagens as a heterogenic protein should be much considered. Lymphocytes are known to have an ability to recognize antigens and facilitate humeral and cellular immunity, making them important for immune response. Therefore, an evaluation on the immunogenicity of tilapia collagen sponges was conducted using a lymphocyte proliferation assay [39]. Mixed lymphocytes were extracted from mouse spleen and seeded onto the tilapia collagen sponges. The results revealed that the tilapia collagens did not trigger lymphocytes proliferation [39]. In addition, an *in vivo* study was also conducted by implanting the tilapia collagen sponges into the subcutaneous tissue of rats [39]. The presence of two major antibodies (IgG an IgM) were detected after 28 days of implantation. However, there was no significant differences in the level of IgG and IgM when compared to the control samples [39]. Therefore, these findings verified the non-immunogenicity responses of tilapia collagens [39].

3.4 Hemostasis

Collagen is a protein which commonly known to have a capability to accelerate blood coagulation by participating in the activation and adhesion of platelets [40]. The blood coagulation process by collagen includes the conversion of fibrinogen into fibrin, capturing platelets and forming clots [41]. As an haemostatic agent, collagen plays a crucial role at wound interfaces by protecting the wound from environmental factors, preserving epithelial cells and increasing the production and permeation of fibroblasts [2]. Amino acid arginine in the structure of collagen is responsible for healing, reducing stress in tissues and increasing the interactions between collagen and platelets [2]. A blood coagulation time *in vitro* is commonly used to measure the clotting capability of biomedical materials.

Previous investigations were performed on tilapia skin collagen sponges to identify their haemostatic properties [25,27]. These sponges were cross-linked with various cross-linking agents where the collagen sponges were reported to have shorter blood coagulation time due to platelet activation by the collagen [25]. However, the cross-linking process did not alter the coagulation time and therefore no difference was spotted between the cross-linked and uncross-linked collagen sponges [25,27]. In

addition, through an *in vitro* hemolysis assay, the blood compatibility of tilapia skin collagen sponges were confirmed to be within an acceptable limit [25,27].

4. Conclusion

Tilapia collagens have suitable chemical and biological properties to be used as biomaterials for TE applications. Higher thermal stability and proline and hydroxyproline amino acid compositions making them as one of the favourable candidate to replace mammalian collagen. In addition, the biocompatibility, biodegradability, immunogenicity and haemostasis properties of tilapia collagen were shown to be comparable to those of mammalian collagen. Therefore, further experiments are needed to explore the potential of tilapia collagen in substituting mammalian collagen for TE applications.

References

[1]Yamada S, Yamamoto K, Ikeda T, Yanagiguchi K and Hayashi Y 2014 *Biomed Res. Int.* **2014** 1–8
[2]R. Valenzuela-Rojo D, López-Cervantes J and I. Sánchez-Machado D 2018 *Seaweed Biomaterials*
[3]Silva J F X, Ribeiro K, Silva J F, Cahú T B and Bezerra R S 2014 *Anim. Feed Sci. Technol.* **196** 96–106
[4]Chen J, Li L, Yi R, Xu N, Gao R and Hong B 2016 *LWT - Food Sci. Technol.* **66** 453–9
[5]Huang C Y, Kuo J M, Wu S J and Tsai H T 2016 *Food Chem.* **190** 997–1006
[6]Li J, Wang M, Qiao Y, Tian Y, Liu J, Qin S and Wu W 2018 *Process Biochem.* **74** 156–63
[7]Potaros T, Raksakulthai N, Runglerdkreangkrai J and Worawattanamateekul W 2009 *Kasetsart J. - Nat. Sci.* **43** 584–93
[8]Noitup P, Garnjanagoonchorn W and Morrissey M T 2005 *J. Aquat. Food Prod. Technol.* **14** 17–28
[9]Ogawa M, Portier R J, Moody M W, Bell J, Schexnayder M A and Losso J N 2004 *Food Chem.* **88** 495–501
[10]Nalinanon S, Benjakul S, Visessanguan W and Kishimura H 2007 *Food Chem.* **104** 593–601
[11]Beil W, Timpl R and Furthmayr H 1973 *Immunology* **24** 13–24
[12]Song W-K, Liu D, Sun L-L, Li B-F and Hou H 2019 *Mar. Drugs* **17** 137
[13]Ellingsworth L R, DeLustro F, Brennan J E, Sawamura S and McPherson J 1986 *J. Immunol.* **136** 877–82
[14]Knott L and Bailey A J 1998 *Bone* **22** 181–7
[15]Sun L, Hou H, Li B and Zhang Y 2017 *Int. J. Biol. Macromol.* **99** 8–14
[16]Zeng S Kui, Zhang C Hua, Lin H, Yang P, Hong P Zhi and Jiang Z 2009 *Food Chem.* **116** 879–83
[17]Zhang Q, Wang Q, Lv S, Lu J, Jiang S, Regenstein J M and Lin L 2016 *Food Biosci.* **13** 41–8
[18]El-Rashidy A A, Gad A, Abu-Hussein A E H G, Habib S I, Badr N A and Hashem A A 2015 *Int. J. Biol. Macromol.* **79** 618–26
[19]Liu H and Huang K 2016 *Int. J. Food Prop.* **19** 63–75
[20]Sujithra S, Kiruthiga N, Prabhu M J and Kumeresan R 2013 *Int. J. Eng. Technol.* **5** 2181–5
[21]Theocharis A D, Skandalis S S, Gialeli C and Karamanos N K 2016 *Adv. Drug Deliv. Rev.* **97** 4–27
[22]Gelse K, Pöschl E and Aigner T 2003 *Adv. Drug Deliv. Rev.* **55** 1531–46
[23]Ikoma T, Kobayashi H, Tanaka J, Walsh D and Mann S 2003 *Int. J. Biol. Macromol.* **32** 199–204
[24]Sugiura H, Yunoki S, Kondo E, Ikoma T, Tanaka J and Yasuda K 2009 *J. Biomater. Sci. Polym. Ed.* **20** 1353–68
[25]Sun L, Li B, Yao D, Song W and Hou H 2018 *J. Mech. Behav. Biomed. Mater.* **80** 51–8
[26]Oh H H, Uemura T, Yamaguchi I, Ikoma T and Tanaka J 2016 *J. Bioact. Compat. Polym.* **31** 31–41
[27]Sun L, Li B, Jiang D and Hou H 2017 *Colloids Surfaces B Biointerfaces* **159** 89–96
[28]Pietrucha K and Safandowska M 2015 *Process Biochem.* **50** 2105–11
[29]Zhang M, Li J, Ding C, Liu W and Li G 2013 *Food Hydrocoll.* **30** 504–11
[30]Huang H N, Chan Y L, Wu C J and Chen J Y 2015 *Mar. Drugs* **13** 2813–33

[31]Ullah S, Zainol I, Chowdhury S R and Fauzi M B 2018 *Int. J. Biol. Macromol.* **111** 158–68
[32]Tang J and Saito T 2015 *Biomed Res. Int.* **2015** 1–8
[33]Pati F, Datta P, Adhikari B, Dhara S, Ghosh K and Mohapatra P K Das 2012 *J. Biomed. Mater. Res. - Part A* **100 A** 1068–79
[34] Nimni M E and Harkness R D 2018 Molecular structure and functions of collagen *Collagen: Volume I: Biochemistry* pp 1–77
[35]Speer D P, Chvapil M, Eskelson C D and Ulreich J 1980 *J. Biomed. Mater. Res.* **14** 753–64
[36]Vizárová K, Bakoš D, Rehákova M, Petríkova M, Panáková E and Koller J 1995 *Biomaterials* **16** 1217–21
[37]Wang J K, Yeo K P, Chun Y Y, Tan T T Y, Tan N S, Angeli V and Choong C 2017 *Acta Biomater.* **63** 246–60
[38]Bentkover S H 2009 *Facial Plast. Surg.* **25** 73–85
[39]Zhou T, Wang N, Xue Y, Ding T, Liu X, Mo X and Sun J 2016 *Colloids Surfaces B Biointerfaces* **143** 415–22
[40]Farndale R W, Sixma J J, Barnes M J and De Groot P G 2004 The role of collagen in thrombosis and hemostasis *J. Thromb. Haemost.* **2** 561–73
[41] Laurencin B D U L S N C T 2011 Biomedical Applications of Biodegradable Polymers *J Polym Sci B Polym Phys. 2011 June 15; 49(12) 832–864.* **15** 832–86480

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