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REVIEW ARTICLE

Genipin-Crosslinked Gelatin Scaffold in Tissue Engineering: A Systematic Review

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ABSTRAK

Gelatin sering digunakan dalam pembuatan kerangka kejuruteraan tisu kerana ia mempunyai ciri-ciri biologi yang baik, termasuk mempercepatkan penyembuhan luka. Genipin, bahan semula jadi yang diperolehi dari tumbuhan Gardenia, terbukti berkesan dalam memperkukuhkan ciri-ciri fizikokimia gelatin. Ulasan sistematik ini melaporkan pengetahuan terkini mengenai pengunaan genipin sebagai agen penyilangan gelatin. Dua pangkalan data elektronik telah digunakan, iaitu Scopus dan MEDLINE melalui Ebscohost. Carian dilakukan untuk penerbitan antara Januari 1999 hingga Disember 2018 menggunakan kata kunci 'gelatin' dan 'genipin'. Makalah berbahasa Inggeris, yang melaporkan kegunaan genipin untuk pembuatan span gelatin telah dipilih. Terdapat 830 makalah dijumpai melalui carian kata kunci di mana 14 makalah telah dipilih dan dibincangkan dalam ulasan sistematik ini. Dapatan kajian termasuk kepekatan, suhu penyilangan, dan cara pembuatan yang optima untuk genipin. Kepekatan genipin yang optima adalah 0.5% dan suhu penyilangan yang optima adalah 25°C. Keputusan kajian ini menunjukkan jurang pengetahuan dalam pengunaan genipin sebagai agen penyilangan gelatin dan lebih kajian diperlukan untuk mengisi jurang ini. Kajian ini menyediakan tinjauan meluas berkenaan pengetahuan terkini mengenai penggunaan genipin sebagai agen penyilangan gelatin.

Kata kunci: fizikokimia, gelatin, genipin, kerangka tisu, kejuruteraan tisu, penyilangan

ABSTRACT

Gelatin has been frequently used in tissue engineering scaffold due to its

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favorable biological properties in wound healing enhancement. Genipin, a natural compound derived from Gardenia plants, was shown to be effective in improving physicochemical characteristics of the gelatin scaffold. This systematic review reported the utility of genipin as a crosslinker in gelatin scaffold fabrication. Two electronic databases, namely Scopus and MEDLINE via Ebcoshost were searched for publication between January 1999 and December 2018, using the keywords 'gelatin' and 'genipin'. Articles published in English, reporting the utility of genipin in the fabrication of gelatin sponge were included. The keywords search yielded 830 articles, in which 14 articles were selected and examined in this review. The result of the search provided input in terms of the optimum concentration, crosslinking temperature, and fabrication method of genipin to be used. From the literature, it was found that 0.5% is the optimum genipin concentration and 25°C is the optimum crosslinking temperature. The result also revealed a gap in the knowledge regarding genipin crosslinker and justifies the need to create awareness of the utility of genipin as a gelatin scaffold crosslinker. The current review provides an extensive overview on the current knowledge on genipin crosslinking and be a guide to an optimal fabrication of the genipin-crosslinked gelatin scaffold.

Keywords: crosslinking, gelatin, genipin, physicochemical, tissue engineering, tissue scaffolds

INTRODUCTION

Injuries or trauma are potentially harmful as they can damage the tissues and lead to tissue degeneration, which entails some kind of intervention to ease its repair, replacement or regeneration (Jammalamadaka & Tappa 2018). There are two types of intervention involves; one that focuses on transplant of tissue from one site to another in a single individual, termed autograft, and another which focuses on the transplant of tissue into the patient from another individual, termed allograft (Vig et al. 2017).

Despite reported efficacy with both intervention type, extensive complications and limitations are still imminent (Buser et al. 2016). Limitation of harvesting autograft is in the context of anatomical constraint, whereby the painful procedure can traumatize the patient, in addition to the monetary cost incurred following management of secondary morbidity that may arise at the donor-site such as infection and hematoma. Alternatively, allografts issues are immune rejection as well as logistical issues such as proper storage and transport, as well as the classic immune rejection by the host problem (Haas et al. 2018; Demetris et al. 2016). However, tissue engineering still plays a crucial role in the regenerative medicine field (Atala 2004; Langer 2000).

Tissue engineering is an interdisciplinary field combining biomedical engineering, cellular and

molecular biology, materials science, and mechanical engineering (Butler et al. 2016). The discovery of tissue engineering was to represent a new scientific field focused on tissue regeneration. The development of tissue engineering has the goal to replace the biological tissues with the support of cells combination and suitable biochemical and physicochemical factors for growth. It generally involves the use of tissue scaffolds for the formation of new viable tissues which is to be applicable for medical purposes. Hence, tissue engineering is the combination of cells from the body and a fabricated scaffold. Ideally the scaffold should have high porosity for cell infiltration, enabling the scaffold to act as template that guide the growth of new viable tissues (Gnavi et al. 2017).

Gelatin is a non-toxic, biocompatible and biodegradable compound with unheard carcinogenicity, that is derived from native collagen undergoing partial hydrolysis. It is a protein that is naturally pure and free from genetically modified organism (GMO) segments (Liu et al. 2015). It does not consist and is totally free of gluten, cholesterol, fat, carbohydrates and also any allergen. Besides, it is a macromolecule that exists with various vital properties. These include the strength of the gel, melting temperatures and the viscosity (Klotz et al. 2016). Additionally, the formation and stabilisation of foams, its pH value and the isoelectric point are of considerable values (Klotz et al. 2016). Gelatin is capable of forming clear solutions that will appear as gel when cooled and melt upon heating. Hence, this statement proves that gelatin is highly viscous. Furthermore, gelatin forms films on the surface and could take in huge water quantity and acts as buffer. It is commercially available at reasonable price and purchasable. It is widely used as a wound bandage materials, scaffolds of tissue engineering and drug delivery carriers that is used broadly in medical field (Zhao et al. 2016). It is used in food and cosmetics as well (Etxabide et al. 2017).

Numerous studies have shown that the application of gelatin sponge is uneasy and tedious although it has been proven to stop the bleeding from punch biopsies. Gelatin can be fabricated into sponge-like 3D structure that can be penetrated with plenty of spaces for the cell to adhere to (Ren et al. 2015). However, gelatin sponge is often observed to be underperforming in terms of mechanical strength and hydrolysis resistance. Crosslinking of gelatin scaffolds with crosslinking materials, stabilized its structure, enforcing their mechanical strength, improve their hydrolysis resistance, increase stability during implantation (Miao et al. 2015; Wang et al. 2015). Crosslinking can occur through physical methods such as ultraviolet radiation and dehydrothermal treatment, or the use of chemical agents like genipin, carbodiimides and glutaraldehyde, or the use of enzymes such as horseradish peroxidases, tyrosinases and transglutaminase.

Meanwhile, genipin, derived from geniposide, is a natural crosslinker that is abundant in Gardenia plants. Genipin was relatively less toxic than

gluteraldehyde (GTA). Genipin reacts with the amino acids in gelatin or collagen to form dark blue pigment, that is used in the fabrication of food dyes (Náthia-Neves & Meireles 2018). Knowledge of genipin on gelatin scaffold tissue engineering is significantly progressing along with many current ongoing studies that is expected to accelerate shortly with effective clinical implementation of gelatin-based products. Thus, in this study, the question on the utility of genipin-crosslinked gelatin tissue engineering was further validated with a systematic review of the literature.

MATERIALS AND METHODS

Search Strategy

A systematic search of the electronic databases was performed to identify relevant studies reporting the utility of genipin crosslink gelatin sponge scaffold in tissue engineering. Two databases were searched in regard to this, Medline via Ebscohost and Scopus (both published between 1999 and December 2018). Two keywords were used in the search strategy; Gelatin* AND Genipin*.

Inclusion and Exclusion Criteria

The results were restricted to only the studies published in English language due to limited resources for translation services. Primary literature with research focus on Gelatin as the only substance that will be crosslinked to form a sponge was also included. Review articles, editorials, news, letter or case studies were excluded from the review. Study not related to Gelatin sponge scaffold were removed.

Data Extraction and Management

Articles underwent screening process prior to their inclusion in this review. Titles and abstracts were screened first to ensure inclusion and exclusion criteria were adhered. Then, the full text of what remaining were read thoroughly and the data extracted. The following information were recorded from the studies: the types of study; aims of study; subject or sample; methods; result; and remarks or conclusion. All the data extraction and management were re-evaluated by two independent reviewers to validate the data integrity.

RESULTS

Literature Search

The keyword search identified a sum of 830 articles across the two databases. All articles were assessed based on the title and abstract by two independent reviewers to ensure compliance to inclusion or exclusion criteria. Both reviewers presented their findings to the third author and underwent rigorous discussion to eliminate bias in selecting the research articles. A total of 525 articles discussed gelatin or genipin individually and were rejected. Another 283 articles were rejected following exclusion criteria namely not in English language, not a primary studies, studying gelatin that was mixed with another substance, and not

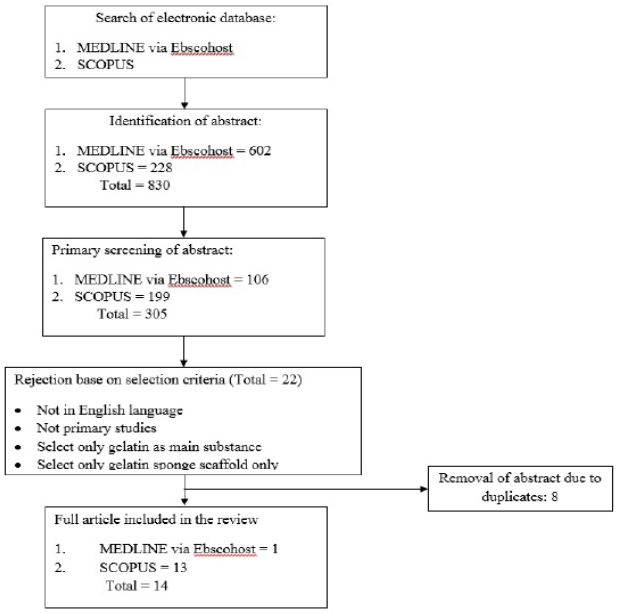

Figure 1: Flowchart of the selction process

related to fabrication of gelatin sponge. All data were extracted directly from the articles. Figure 1 described the flow chart of the selection process including reasons for exclusion. Further details on each study regarding methodological and outcome aspects were summarised in Table 1.

Study Characteristics

All studies included in this review were published between the year 2006 and 2018. All studies included report on the different fabrication methods of genipin-crosslinked gelatin scaffold. Three studies investigated the effect of using different concentration of genipin (Sánchez et al. 2017; Amadori et al. 2015; Chang 2009), three studies investigated the effect of different crosslinking temperature (Thakur et al. 2012; Lien et al. 2010; Lien et al. 2009), one study each investigated alternative freezing (Saglam et al. 2013), drying (Liu et al. 2008), and crosslinking method (Lien et al. 2008), three studies compared genipin and other types of crosslinker (Yang et al. 2018; Poursamar et al. 2016; Tonda-Turo et al. 2011), and one study compared between a porous and non-porous scaffold (Chang et al. 2009). Another study included in this review, compared the biocompatibility of their scaffold with different cells without any difference in the fabrication method.

Fabrication of Gelatin Scaffold with Different Concentration of Genipin

The concentration of the genipin used during crosslinking determines the porosity of the scaffold. Among the concentrations used in the studies are 0.1, 0.2, 0.3, 0.5, 2.5, 5, 10, and

Table 1: Summary of the study which genipin is included

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
1	Yang et al. 2018	4%	15 mM	Treatment group: 1) Gelatin sponge crosslink at 37°C Parameter: 1) Porosity was evaluated through liquid displacement method. 2) Mechanical testing was systematically investigated on a uniaxial mechanical testing apparatus (HPB, Handpi, China) equipped with 20N capacity. 3) Swelling ratio was evaluated by immersing gelatin sponge into deionized water at room temperature for 1h. 4) Degradation rates of gelatin sponges was evaluated by exposing them to various enzymes.	1) pore size of genepin (GP)-sponge varies signifcantly, and the average porosity is 66.6±5.3% 2) The modulus of GP-sponge when dry is 964.2±42.0 kPa and when wet is 261.4±13.9 kPa 3) GP crosslink sponge exhibit 939.5±20.7% swelling ratio 4) Enzymolysis rate of collagenase on GP-sponge is markedly slower than those of mTG-sponge and EDC-sponge	GP-sponge exhibits good resistance to hydrolysis and enzymolysis compare to other crosslinker. GP-sponge is a suitabe biomaterial for hard tissue repair, as it has lower water absorption, larger pore size and higher mechanical strength.
2	Sánchez et al. 2017		0.1, 0.2, 0.3% (w/v)	Treatment group: 1) Gelatin sponge crosslink at 25°C Parameter: 1) Swelling ratios were determined using a gravimetric method 2) Mechanical properties were evaluated and calculated following the protocol described by Acosta Santamaría 3) Cell viability was measured via MTT assay	1) Swelling ratio decreased with increase concentration of genipin a) 907% at 0.1% concentration b) 692% at 0.2% concentration c) 604% at 0.3% concentration 2) Young´s modulus increased along with concentration of genipin a) 51.69 kPa at 0.1% concentration b) 81.16 kPa at 0.2% concentration c) 90.29 kPa at 0.3% concentration 3) Cell survival rate was improved at 0.1%, 0.2%, and 0.3% genipin concentration. Maximum viability was observed at 0.3% concentration	Higher genipin concentration, lower swelling ratio. Higher genipin, higher young modulus.

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
3	Poursamar et al. 2016	20% w/v	0.5 mol/v	Treatment group: 1) Gelatin sponge crosslink at 25°C Parameter: 1) Mechanical testing was carried out using a texture analyzer (TA.XT-Plus, Stable Micro Systems, UK) 2) Swelling ratio was evaluated by immersing gelatin sponge into deionized water at 4°C 3) The average pore sizes of the scaffolds were determined using Quartz PCI image processing software package (Quartz Image Corp., Vancouver, Canada) 4) Cell viability was measured via MTT assay	1) Genipin-crosslinked samples had a Young's modulus of 9.3 kPa 2) Genipin crosslink sponge exhibit 440% swelling ratio 3) Genipin crosslink sponge exhibit 520 (±163) μm pore size 4) Fibroblast cell can survive under 0.5 mol/v genipin crosslink concentration	Cell viability for the genipin-crosslinked scaffolds was significantly higher than the other crosslink samples; however in term of pore structure, the genipin crosslinked samples showed more distorted porosity.
4	Amadori et al. 2015	10% wt/V	0.15% wt/V	Treatment group: 1) Gelatin sponge crosslink at 37°C Parameter: 1) To measure the equilibrium Water Uptake Ability of the scaffold, pre-weighed dry sample was immersed in PBS for 20s 2) Compression tests were performed on 1 x 1 x 1 cm samples using a 4465 Instron testing machine, equipped with a 1 kN load cell 3) For quantitative three-dimensional (3D) analysis of the material porosity, the samples were scanned using a high-resolution microCT system SkyScan 1172 (Bruker Micro-CT, Kontich, Belgium 4) Cell viability was measured via MTT assay	1) Genipin crosslink sponge exhibit 4318 ± 88 % water uptake ability 2) The elastic moduli of genipin crosslink gelatin is 1.1 ± 0.4 mPa 3) Genipin crosslink sponge exhibit 93.44 ± 0.16 % porosity 4) MTT assays revealed no toxicity effect towards all groups	GP-sponge show good viability towards chondrocytes cells

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
5	Focaroli et al. 2014	10 wt%		Treatment group: 1) Gelatin sponge crosslink at 40˚C for 5 minutes Parameter: 1) Cell viability was measured via MTT assay	1) hADSCs cell can survive under 0.15% wt/V genipin crosslink concentration	GP-sponge show good viability towards hADSCs
6	Saglam et al. 2013	2.50%	0.22/44 mM	Treatment group: 1) Gelatin sponge crosslink at 25˚C for 48 hours Parameter: 1) Mechanical properties were determined using a Bohlin parallel-plate Rheometer (Malvern Instruments Ltd, Worcestershire, UK) 2) Pore size were evaluated using Zeiss Supra 50VP (Thornwood, NY) SEM	1) The elastic moduli increased with increasing genipin concentrations, ranging from ~1.5 to ~51 kPA 2) Pore size increase from ~100 to ~300 m as the genipin concentration is increased from 0.22 to 44 mM	Higher GP concentration, higher elasticiy. Higher GP concentration, higher pore size.
7	Thakur et al. 2012	10% (w/w)	0.4% (w/v)	Treatment group: 1) Gelatin sponge crosslink at 5, 15, 25˚C for 24 hours Parameter: 1) Pore size were evaluated using a scanning electron microscope (SEM) (JEOL, JSM-6380LV) 2) The equilibrium water content (EWC) of the hydrogels was measured after 24 h of swelling in deionized water 3) Crosslinking density were evaluated as described by Sen et al., 1999	1) Gelatin crosslinked at 5˚C have the largest pores a) 5˚C (~45 µm) b) 15˚C (~36 µm) c) 25˚C (~15 µm) 2) Gelatin crosslinked at 5˚C showed highest EWC a) 5˚C (~969%) b) 15˚C (~805%) c) 25˚C (~704%) 3) Gelatin crosslinked at 25˚C showed the highest crosslinking density a) 25˚C (88.01±1.47) b) 15˚C (76.49±0.40) c) 5˚C (20.48±0.50)	Degree of crosslinking was dependent on crosslinking temperature. Between 5, 15 and 25˚C, 25˚C is the best temperature to crosslink.

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
8	Tonda-Turo et al. 2011	2.50%	2.5%(w/w)	Treatment group: 1) Gelatin sponge crosslink with 2.5% (w/w) Parameter: 1) The swelling ratio were evaluated using a phosphate buffered saline (PBS) at pH 7.4 (Sigma-Aldrich) 2) Mechanical characterization were measured using MTS QTest/10 device and a load cell of 500 N 3) Pore dimension was quantified by analyzing the SEM images of fractured sections using an image software (ImageJ 1.43)	1) Genipin crosslink sponge exhibit 992±52% swelling ratio 2) The elastic moduli increased when crosslink with genipin a) Non crosslink (0.50±0.18 MPa) b) Genipin crosslink (1.20±0.29 MPa) 3) Genipin crosslink sponge exhibit 120μm pore size	Gelatin crosslink with genipin increase its mechanical properties.
9	Lien et al. 2010	5 wt.%	0.5 wt.%	Treatment group: 1) Gelatin sponge crosslink at 10, 15, 20, and 25°C Parameter: 1) Pore size were evaluated using scanning electron microscopy (SEM, JEOL JSM-5600)	1) Scaffold crosslinked at lower temperature has smaller pores a) 10°C (50-150 μm) b) 15°C (100-200 μm) c) 20°C (250-350 μm) d) 25°C (350-500 μm)	Different crosslink temperature affect pore size.
10	Chang et al. 2009	3% (w/w)	0, 0.1, 0.5, 1.0, 1.5% (w/w)	Treatment group: 1) Gelatin sponge crosslink at 0, 0.1, 0.5, 1.0, 1.5 % (w/w) for 7 days Parameter: 1) Cell viability was measured via MTT assay	1) MTT assays revealed no toxicity effect towards all groups	Genipin crosslink gelatin does not have any cytotoxic effect towards PC12 cells

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
11	Chang et al. 2009	10% (wt/wt)	0.5% (wt/wt)	Treatment group: 1) Gelatin sponge crosslink for 48 hours Parameter: 1) Porosity of the scaffold was calculated using density displacement method 2) Mechanical properties were determined using (AG-IS, Shimadzu Co., Kyoto, Japan) 3) Swelling ratio were evaluated using deionized water at room temperature 4) Cell viability was measured via MTT assay	1) Genipin crosslink sponge exhibit 90.8±0.9µm porosity 2) Genipin-crosslinked samples had a Young's modulus of 0.94±0.22 MPa 3) Genipin crosslink sponge exhibit 120% swelling ratio 4) Schwann cells can survive under 0.5% (wt/wt) genipin crosslink concentration	Genipin crosslink gelatin does not have any cytotoxic effect towards Schwan cells
12	Lien et al. 2009	5wt.%	0.5 wt.%	Treatment group: 1) Gelatin sponge crosslink at 10, 15, 20 and 25°C for 48 hours Parameter: 1) Crosslinking degree was determined by the ninhydrin assay 2) Pore size were evaluated using a JEOL JSM-5600 microscope 3) Swelling ratio were determined by soaking in PBS (diluted from Dulbecco's phosphate-buffered saline 10, Biowest)	1) Scaffold crosslinked at lower temperature has lower crosslinking degree a) 10°C (47%) b) 15°C (48%) c) 20°C (59%) d) 25°C (63%) 2) Scaffold crosslinked at lower temperature has smaller pores a) 10°C (50-150 µm) b) 15°C (100-200 µm) c) 20°C (250-350 µm) d) 25°C (350-500 µm) 3) Scaffold crosslinked at lower temperature has lower swelling ratio a) 10°C (310%) b) 15°C (370%) c) 20°C (490%) d) 25°C (495%)	Different crosslink temperature affect crosslinking degree, pore size and swelling ratio.

No	Articles	Concentration of gelatin	Concentration of genipin	Methodology	Results	Conclusion
13	Liu et al. 2008	10%	0.5% (w/v)	Treatment group: 1) Gelatin sponge crosslink for 72 hours Parameter: 1) Swelling ratio were determined by soaking in Ringer solution at 37°C.	1) Genipin crosslink sponge exhibit 2.45% swelling ratio	0.5% (w/v) genipin crosslink gelatin (10%) exhibit low swelling ratio
14	Lien et al. 2008	5, 7.5, 10 wt.%.	0.5 wt.%	Treatment group: 1) Gelatin sponge crosslink at 25°C for 48 hours a) Method I (the mixing-crosslinking method) b) Method II (the scaffold-crosslinking method) c) Method III (the gel-crosslinking method) Parameter: 1) Crosslinking degree was determined by the Ninhydrin assay	1) The extents of crosslinking of the scaffolds are roughly the same at about 85% except that of scaffold made through method I with gelatin contents of 7.5 and 10 wt.% at 82% 75%	Method of crosslinking may affect the crosslinking degree of the scaffold

20% weight per volume of genipin. In this review, consensus genipin concentration was 0.5% weight per volume.

As the concentration of genipin increase, more molecules of the crosslinker are available to bridge the gap between adjacent gelatin molecules. This result in higher crosslinking degree that can reach to a fully solid structure. By reducing the concentration, degree of the crosslinking reduces, allowing for formation of a porous structure.

Sanchez et al. (2017) investigated the effect of different genipin crosslinker concentration on the water uptake and tensile strength of their gelatin scaffold. When genipin concentration was higher, the resulting scaffold had lower swelling ratio. Alternatively, higher tensile strength was detected with higher concentration of genipin (Sanchez et al. 2017).

Fabrication of Gelatin Scaffold with Different Crosslinking Temperature of Genipin

The temperature change during crosslinking of the gelatin scaffold can affect the pore size. This is due to the ice crystal formation within the unpolymerized gelatin gel. Gelatin crosslinking with genipin was done at the temperature of 5, 10, 15, 20, 25°C. In this review, consensus on the optimum crosslinking temperature was 25°C.

Pore size of 350-500m is desirable in the fabrication of articular cartilage engineered tissue to allow cell population of chondrocyte into

the gelatin scaffold. Crosslinking temperature of 25˚C, not only resulted in good crosslinking degree and swelling ratio (Lien et al. 2009), but also resulted in the formation of the appropriate pore size for articular cartilage engineering with favorable compression strength (Lien et al. 2010). Thakur et al. (2012) also supported the optimum crosslinking temperature of 25˚C upon measuring their scaffold's pore size, swelling potential, and crosslinking degree following fabrication with crosslinking temperature of 5, 15, and 25˚C. As the temperature decreased, scaffold pore size, water uptake and crosslinking degree increased, reaching to the level that hinders its utility in the controlled release of indomethacin. Hence, they concluded that 25˚C, their highest crosslinking temperature as their most optimum crosslinking temperature (Thakur et al. 2012).

Different Fabrication Method of Genipin-Crosslinked Gelatin Scaffold

Scaffold fabrication generally involves four steps, mixing of the biomaterial and its crosslinker, polymerization, freezing, and drying until it forms the spongy scaffold (Method 1). Other than being added together into the mixture of biomaterial, genipin crosslinker can also be added after freeze-drying of the scaffold (Method 2) or following gelation of the gelatin (Method 3). When comparing the crosslinking degree between the three aforementioned methods, Lien et al. (2008) found no significant different among the resulting scaffold. However, method 1, which is considered as traditional method for fabricating genipin-crosslinked gelatin, demonstrated a lower crosslinking degree at 82% and 75% for gelatin concentration of 7.5 and 10% respectively, compared to the about 85% for all other scaffold groups in their study (Lien et al. 2008). This suggest effect of the fabrication method in crosslinking degree of the scaffold.

To form a gelatin sponge, a drying method need to be employed on the gelatin hydrogel. The gold standard for fabrication of gelatin sponge for drug or protein delivery is the freeze-drying method, using a vacuum to absorb moisture from a frozen material. A study that compares different drying methods; oven drying, room temperature drying, and freeze-drying, revealed the importance of freeze-drying in forming porous gelatin scaffold. Oven and room temperature drying were unable to form porous structure despite their superior drying rate and tensile strength. This shows the utility of freeze-drying method in fabricating porous gelatin scaffold for tissue engineering application (Liu et al. 2008).

In this review, studies that look into alternative freezing method were also included. Traditionally, gelatin gel is frozen in a mold, resulting in a random direction heat transfer within the scaffold. Saglam et al. (2013) introduces a specialised freeze-casting device that resulted in unidirectional heat transfer during freezing. The unidirectional freezing cause the formation of longitudinal

channels as opposed of random pores in the traditional freezing method. The unidirectional frozen scaffold, demonstrated superior elasticity compared to the traditional gelatin scaffold (Saglam et al. 2013). Their purpose of fabricating longitudinal channels is to guide aligned axonal connections throughout the injured tissue in nerve tissue regeneration.

Fabrication of Gelatin Scaffold with Different Crosslinker

According to the literature, three studies used different chemical crosslinker to fabricate their scaffold including genipin, glutaraldehyde, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), hexamethylene diisocyanate (HMDI), poly (ethylene glycol) diglycidyl ether (epoxy), and glycidoxypropyltrimethoxysilane (GPTMS). One study also included the enzymatic crosslinker microbial transglutaminase (mTG). Scaffold characteristics such as porosity, mechanical strength, swelling ratio, enzymatic degradation, pore size, and thermal properties were reported among all studies included (Yang et al. 2018; Poursamar et al. 2016; Tonda-Turo et al. 2011).

DISCUSSION

The utility of genipin-crosslinked gelatin scaffold is tremendous. From the findings in this review, the scaffold in question was intended for nerve tissue regeneration (Saglam et al. 2013; Chang 2009; Chang et al. 2009), controlled release drug delivery (Thakur et al. 2012), and articular cartilage tissue engineering (Lien et al. 2010; Lien et al. 2009; Lien et al. 2008). The great utility of genipin-crosslinked gelatin scaffold is due to the ease in manipulating the physical properties of the final scaffold during fabrication. Hence, knowledge on the genipin-crosslinked gelatin fabrication such as crosslinker concentration, crosslinking temperature, freezing method and drying method on the physical parameter outcome of the finished scaffold is important.

In this review, we compiled all available report on genipin-crosslinked fabrication to compare and contrast the genipin concentration, crosslinking temperature, and freeze-drying method that each researcher used. The fourteen articles retrieved from the systematic search of the literature suggest the scarcity of the knowledge on genipin-crosslinked fabrication. This finding emphasized the need of study concentrating on the different fabrication method of genipin-crosslinked gelatin scaffold to further understand the effect of different fabrication on the scaffold utility.

The literature search revealed Lien et al. (2008) as the earliest study that reported on the fabrication of genipin-crosslinked gelatin scaffold. They compared the three method that differs on the step where crosslinker was introduced. Crosslinker can be introduced by mixing with gelatin before gelation, after gelation, or after freeze-drying. Although no difference was found in terms of crosslinking degree between the different method, most of the study included in this

review, introduced their crosslinker before the gelation of gelatin (Lien et al. 2008).

Another pioneering study in genipin-crosslinked gelatin scaffold, is Liu et al. (2008). They compared the different drying temperature in order to fabricate dried gelatin sponge for the delivery of lyophilised protein in clinical setting. They demonstrated the importance of freeze-drying technique in order to fabricated a porous scaffold. Until today, freeze-drying method has been considered the gold standard for drying as evidence by our literature findings. All of our included studies employed freeze-drying to obtained their porous gelatin scaffold (Liu et al. 2008).

The use of 0.5% genipin concentration was reported in six out of fourteen studies included in this review (Lien et al. 2010; Chang 2009; Chang et al. 2009; Lien et al. 2009; Lien et al. 2008; Liu et al. 2008). This implied the consensus of 0.5% as an optimum concentration for the genipin crosslinking. With 0.5% genipin concentration, it has been reported that swelling ratio were between 120-495%, porosity at around 90.8%, pore size between 50-500m, tensile strength of 0.94mPa, and crosslinking degree between 47-85%. Decreasing the concentration of genipin will result in increment of the swelling ratio and pore size while reducing tensile strength, which can be seen in 0.15% genipin (Amadori et al. 2015).

In terms of crosslinking temperature, the use of the room temperature, 25˚C in seven studies included in this review implied its advantage as optimum crosslinking temperature (Sánchez et al. 2017; Poursamar et al. 2016; Saglam et al. 2013; Thakur et al. 2012; Lien et al. 2010; Lien et al. 2009; Lien et al. 2008). With 25˚C crosslinking temperature, a favorable scaffold size, 350-500m can be achieved. Crosslinking at lower temperature than 25˚C, resulted in lower pore size, crosslinking degree, and swelling ratio. The opposite can be observed with crosslinking at higher temperature where there was an increase in pore size, crosslinking degree, and swelling ratio reported.

Conducting the current systematic review was not without limitation. Due to its great utility and versatility, selecting keywords to be used in the electronic database search was difficult. In order to ensure sensitivity, the authors decided to use the two keywords that represent the research question. Limited keywords are not typical in a systematic review as it can sacrifice specificity of the search. However, the literature search were still able to identify available study on the utility of genipin.

CONCLUSION

The studies included in the review, provide insight in selecting the fabrication parameters to produce genipin-crosslinked gelatin tissue engineering scaffold with great utility. Moreover, the findings from this study can be a guidance for further study in genipin-crosslinked gelatin scaffold fabrication to improve accumulated knowledge of genipin-crosslinked gelatin scaffold in tissue engineering.

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