Skin Tissue Engineering

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Skin tissue engineering aimed to replace chronic tissue injury commonly occurred due to severe burn and chronic wound in diabetic ulcer patients. The normal skin is unable to be regenerated until the seriously injured tissue is disrupted and losing its function. 3D-bioprinting has been one of the effective methods for scaffold fabrication and is proven to replace the conventional method, which reported several drawbacks. In light of this, researchers have developed a new fabrication approach via 3D-bioprinting by combining biomaterials (bioinks) with cells and biomolecules followed by a suitable crosslinking approach. This advanced technology has been subcategorised into three different printing techniques including inject-based, laser-based, and extrusion-based printing. However, the printable quality of the currently available bioinks demonstrated shortcomings in the physicochemical and mechanical properties.

3D-bioprinting natural-based bioinks wound healing skin regeneration 3D-printing quality

1. Introduction

Skin injury has become a significant problem that can cause impairments to the patients' quality of life [1]. A skin injury can be classified based on two different categories, which are acute and chronic wounds. An acute wound is usually able to recover within the wound healing time frame. There are several types of chronic wounds including wound infection, diabetic ulcer, and gangrene [2]. In 2018, Medicare beneficiaries identified 8.2 million patients with open wounds with or without infections in which this number is estimated to increase in the future [3]. In Malaysia, diabetic foot ulcers have become a significant concern among healthcare workers because of the prevalence of diabetes mellitus (DM) patients increases every year. These diabetic patients are prone to have chronic diabetic foot ulcers that are severe and involving a long-term impact on their lives [4].

Worldwide, diabetes has become a common disease with increasing cases daily. Based on the data reported by the National Diabetes Registry (NDR) by our Ministry of Health (MOH) Malaysia, the number of diabetic patients that have successfully registered by NDR was 1,614,363. This is targeted to increase in the future [5]. Furthermore, in the United States of America (USA), 6.5 million people are severely affected by chronic wound infections followed by an increasing number of diabetic patients with diabetic foot ulcers [6].

The National-Health Morbidity Survey (NHMS) reported that the prevalence of the diabetic burden in Malaysia increased from 15.2% in 2011 to 17.5% in 2015 [7]. The following statistics indicate that the prevalence of diabetes

has increased approximately 14% within 5 years. An increasing number of diabetic patients reflects the increasing demand for wound-dressing supplies.

The Ministry of Health (MOH) Malaysia has a proper wound care guideline to handle wound injury. Wound care approaches are usually based on wound characteristics and assessments. Any wound exposed to infections will be prescribed antibiotics to stop the infection. Several types of wound dressing are available for wound treatment including hydrogel, hydrocolloid, alginates, foams, and films. The goals for each wound dressing are to maintain the wound's environment, prevent infections, and minimise skin irritation [8]. Other than wound dressing, tissue engineering has been widely used and practised clinically to replace injured tissue due to chronic wound and promotes skin regeneration.

The application of tissue engineering has already been explored a long time ago using several conventional fabrication techniques. However, for chronic wounds, immediate treatment and tissue replacement are needed to avoid prolonged exposure to the environment. In skin tissue engineering, a 3D-shaped scaffold that has been seeded with cells is used to maintain the tissue homeostasis process [9].

A wound that is exposed to the environment is prone to get wound infections and complications. Therefore, 3D-bioprinting has been introduced to overcome the drawbacks of the conventional method especially related to production time. 3D-bioprinting has a high potential to deliver immediate treatment to the patient and plays a significant role in rapid treatment to promote skin regeneration and wound healing.

2. Factors That Affect Low Printability Quality in 3D-Bioprinting

The 3D-bioprinting technique is very challenging due to its printing issues that affect the scaffold's printability quality. The printability can affect the gross appearance, morphology, and mechanical properties of the scaffold [10]. Several factors can influence the printability quality of 3D-bioprinting including the type of printing method, type of bioinks, the viscosity of the hydrogel, shear-thinning property, scaffold porosity, and structural fidelity. All of these printability factors are summarised in Table 1.

Table 1. The factors that were affected by low printability quality in 3D-bioprinting technique.

Bioinks	Printing Method	Factors that Affected by Low Printability Quality				Strategies to Improve Printability	References
		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
Hydrogels	Extrusion-based bioprinting Lithography-based bioprinting	Higher viscosity of the hydrogel will result in high printing fidelity.	Shear stress increases due to high viscosity of hydrogels.	The thickness of the hydrogel layers may influence the	Cross-linker efficiency and structural stability for postprinting.	The optimal temperature of each hydrogel must be identified because it has influenced	[11][12]

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		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
				size of the pores.		viscosity of the hydrogels. Increase printing resolution for shape fidelity. Hydrogels must be physically or chemically crosslinked to facilitate the shape of the 3D-structure. Several printing patterns were suggested to enhance pore structures, including zigzag and honeycomb patterns.	
Alginate-Gelatin	Extrusion based bioprinting	High viscosity of alginate-gelatin bioinks promotes unstable and irregular forms of hydrogels during printing. The viscosity of the alginate-gelatin bioinks is influenced by the temperature of the gelatin to become gel and solid. The higher viscosity of gelatin will result in higher	Not-Reported	Not-Reported	Alginate and gelatin have low structural fidelity. Loss modulus of the alginate will negatively affect the shape fidelity of the printed hydrogel.	The concentration of gelatin must be higher than alginate to ensure right viscosity and storage modulus. The optimum printing temperature for alginate-gelatin is between 20–25 °C. Alginate known as low bioadhesivity bioinks. Therefore, alginate need to be used with gelatin to provide the ligands for cell attachments and mimics the native ECM. The covalent crosslinking technique should be used to enhance the	[12][13][14][15][16][17]

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		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
		modulus storage. Besides, the higher viscosity of alginate will increase in loss modulus.				mechanical properties of alginate. The printability quality of alginate-gelatin bioinks can also be supported by the addition of an extruder heating system.	
Agarose-Collagen	Extrusion-based bioprinting	Collagen has low viscosity and slow gelation time. Agarose has rapid gelation time and its viscosity influenced by the temperature.	Not Reported	Not Reported	Agarose supports the mechanical strength of the collagen bioinks.	Collagen type I needs to be used with agarose to enhance the viscosity, gelation time, and support the mechanical strength. The strategies to improve shear thinning and porosity structure for agarose-collagen bioinks are not reported.	[12][18]
Chitosan-Gelatin	Extrusion-based bioprinting	The viscosity increased as the concentration increases.	Flow rate increased according to the diameter of the nozzle	Chitosans have shear thinning behavior.	Chitosan-gelatin hydrogel has excellent mechanical strength.	Appropriate concentrations of the chitosan-gelatin bioinks should be used since they have influenced the viscosity of the hydrogels. The optimum size of the nozzle is necessary to monitor the printing of the hydrogel. Chitosan must be combined with other natural biomaterials for better mechanical stability.	[12][19][20]

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Cellulose-Alginate	Extrusion-based bioprinting	A lower viscosity of alginate will disrupt cell viability.	Not Reported	Not Reported	Not Reported	The combination of alginate with nanofibrilated cellulose (NFC) resulting an excellent 3D printing.	[12]
Silk fibroin-Gelatin	Extrusion-based bioprinting	The viscosity of silk fibroin influenced by the temperature.	Exposure of shear force >100 s^{-1} towards silk fibroin bioinks during printing results in nozzle clogging.	Have interconnected pore structures that enable cellular migration activity.	Printed hydrogels that are made up of silk have high compatibility with high structural fidelity.	Mix homogeneous living cells before printing process to allow easy mixing and achieve optimal viscosity without affecting cell viability. Apply low shear force (<100 s^{-1}) during printing to reduce shear rate. The printed hydrogel can be deposited in 80–90% of alcohol to permit a faster solidification. However, this is not suitable with cells. Silk fibroin need to combine with gelatin bioinks to produce putative cell attachments motifs.	[21][22][12][23][24]
Gelatin-Elastin	Extrusion-based printing	The viscosity of the gelatin-elastin bioinks depending on the adjusted temperature.	Shear stress increased from 0.79 to 1.17 kPa when the extrusion pressure increased from 5 kPa to 25 kPa	Not-Reported	Construct with a complex architecture shape of the scaffold will improve the printing fidelity.	Handle with a temperature of 8 °C for optimum viscosity. The final printing condition was selected as 15 kPa pressure and 30 mm s 1 at 8–10 °C, resulting in 1.08 kPa shear stress.	[14][15]

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		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
						Used cold water fish gelatin to enhance the printability of bioinks. Crosslinking with visible light is required to enhance the mechanical strength of the hydrogel. Strategies to enhance porosity structure for gelatin-elastin hydrogels are not reported.	
Alginate-Honey	Extrusion-based bioprinting	The use of alginate alone tends to be high in viscosity and therefore difficult to print.	High viscosity of alginate induces shear thinning during the printing process.	Alginate hydrogel has low porosity structure.	Low shape fidelity.	Use honey as natural materials/remedies to reduce the viscosity of alginate, improve the structural fidelity of the printed hydrogel, and increase the gelation time. Use up to 5% concentration of honey to retain the porous structure of the printed hydrogel. Strategies to improve shear thinning for alginate-honey bioinks are not reported.	[16]
Alginate	Extrusion-based bioprinting	The viscosity of alginate bioinks influenced by the amount of	Not Reported	High porosity of hydrogel structure.	Not Reported	Choose the right size of nozzle/valve for printing because it affects cell viability	[25][17]

3. Conclusions and Future Perspectives

Bioinks	Printing Method	Factors that Affected by Low Printability Quality				Strategies to Improve Printability	References
		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
		alginate powder and suitable temperature use.				and shear thinning rate. Alginate bioinks suitable to perform physical crosslinking to enhance shape fidelity.	
Gelatin Methacrylate (GelMA)	Extrusion-based bioprinting	The adsoption of GelMA towards nanocellulose has impacts on the viscoelasticity of the hydrogel and it becomes easier for the hydrogel to move out from the nozzle.	Nanocellulose shows shear-thinning behavior.	Not Reported	The incorporation of GelMA with nanocellulose increased the solid content of the bioinks. Therefore, it will increase the shape fidelity of the hydrogels.	Adjusted the printing parameters based on viscoelasticity of bioinks. Used 2000 mm/min of printing speeds. Combine GelMA bioinks with nanocellulose to enhance mechanical strength of the hydrogel.	[26]
Furfuryl-Gelatin	Extrusion-based bioprinting	Insufficient viscosity for printing.	Insufficient shear thinning.	Have adequate porosity structure for cellular activity.	Low structural fidelity.	Addition of a small quantity of hyaluronic acid (HA) to enhance the viscosity of the hydrogel. Strategies for managing shear thinning are not reported. Requires crosslinking with visible light to achieve good structural fidelity.	[27]
Collagen	Extrusion-based bioprinting	Low viscosity	Increase in shear rate	The usage of collagen bioinks without a crosslinker does not produce a	Weak mechanical strength.	Use of low pH, mild collagen composition showed dense collagen fibers with a large pore	[28][29]

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		Viscosity of Hydrogel	Shear-Thinning Property	Scaffold Porosity	Structural Fidelity		
				porous structure of hydrogel.		size. Print collagen bioinks below gelation time (35 °C) to prevent shear stress. 5% collagen is the optimum concentration to reduce shear stress and for high cell viability. Crosslink the collagen bioinks with a crosslinker (physical or chemical), or can use with other biomaterials including natural and synthetic polymers to enhance mechanical strength of the hydrogels.	

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