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REVIEW ARTICLE

Tissue Engineering in Wound Healing Therapy by Using Chitosan and Stem Cells

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Abstract

Skin wounds are considered as the most critical, and widespread problems due to the high rate of accidents or burn worldwide. Full-thickness skin loss could be chronic or acute wounds**,** which found difficulty in medical treatment, because of taking a long time in healing. Therefore, exposure to the infection, which delays the healing process and causing disordered healing, ends with distortion or incomplete closure, which might lead to a fatal complication. Thus, the need for skin grafting is a necessary and critical decision although its side effects and rejection possibilities. This review put a spotlight on one of the latest therapeutic techniques in regenerative medicine "tissue engineering". It is an up-to-dates field in applications of stem cells and cells culture to replace the traditional tissue or organs transplantation techniques. We discussed the skin organ scaffolding as a simple example of tissue engineering applications in accelerating wound healing using stem cells cultured with chitosan scaffold. The latter is characterized by several medical properties especially in skin regeneration, for fabrication of artificial skin, which mimics the natural skin tissue and applying it in chronic or acute wounds therapy.

Keywords: Wound healing, Tissue engineering, Stem cells, Chitosan, Scaffold.

Introduction

Organ transplantations take increased attention in recent years, throughout reconstructive surgery and prosthetics for treating damaged organs or tissues and also post amputation and surgical resection [1].

The disadvantages of traditional techniques drive the scientists for striving towards tissue engineering therapy (TE). It is a novel advanced technique that combines the engineering and cell biology field to manufacture a biological scaffold. This mimics the living organ's function and anatomical structure to be suitable for transplantation instead of damaged organs or tissues, such as heart, kidney, bone, and skin [2]. The basic tools of tissue engineering techniques are considered as cells and substance making the scaffold with additional enhancer tools such as growth factors. Tissue-engineered organs are manufactured of autologous cells, which are biocompatible and keep tissue function that aids the damaged organ to regenerate into neo-tissue organs. It is considered a potential therapeutic advantage to avoid the defects resulted from organs transplantations such as immune rejection [3].

The scaffold is a three-dimensional (3D) structure where cells are seeded in it to mimic the natural environment, estimate cellular adhesive and proliferative properties, cells migration, and gas exchange. In addition, one of most potential features of the 3D structure is the rate of degradation, which is proportional with tissue formation rate [4].

The scaffold could be manufactured from natural polymers such as chitosan, cellulose, collagen, and gelatin [5- 9] or synthetic polymers such as poly caprolactone (PCL), poly L-lactic acid (PLLA), poly glycolic acid (PGA), and poly lactic-co-glycolic acid (PLGA) [10, 11].

Several techniques could be applied for scaffold fabrication such as freeze-drying, electrospinning, decellularization, and 3D printing [12].

Chitosan is a derivative of chitin, which is considered the second most abundant biopolymer after cellulose in nature. Chitin is the main content of the exoskeleton of crustaceans and insects [13].

Chitosan has several medical applications due to the positively charged amino groups, such as immobilization of cells and enzymes, drug carrier, biodegradable films and fibers, contact lenses, wound suture, and artificial skin [14, 15]. Chitosan is non-toxic, biodegradable, with anti-microbial activity, and biocompatibility, and due to its medical properties. Moreover, chitosan enrolled in many applications in accelerating wound healing [16].

Stem cells are considered non-differentiated cells, which exist in embryonic tissues, and in various adult tissues of the body. These cells could be differentiated into several cell types, and are characterized by self-renewal [17] and potency, which is the ability and tendency to differentiate into several types of cells [18]. Stem cells can be obtained from various sources such as embryonic tissue, adipose tissue, and umbilical cord blood [19, 20].

The wound is a cut or damage that induces injury on the skin. Wound healing is a process of cascaded series of the following phases: hemostasis, inflammation, proliferation, and remodeling, which end with recovering skin integrity. In chronic wounds, it was noticed that the healing cascade phases are not completed, therefore the wound is more exposed to tissue necrosis through the second infection, which results in an increased number of exudates and pus cells number [21]

In this review, we will discuss the use of scaffolds as biological substitutes and stem cells in skin tissue regeneration.

Skin role as a barrier and wounds complications

The skin acts as a barrier and protects the body from penetration of foreign organisms, harmful substances, and water loss, regulates the temperature, and connects the living body with the surrounding environmental conditions. The skin can be exposed to damage caused by chemical or mechanical damage and microorganisms [22].

Wounds have got great attention because they risked causing infections, high cost of treatment by drugs, and sometimes causing death in severe cases. Wound infection or inflammation can come from surgical interventions, abrasions, trauma, or burns, and if not properly treated can trigger sepsis and cause death [23, 24].

Skin anatomy and structure

The skin is an integument, which is considered as the largest organ that covers the whole external surface of the body. It is divided into thin and thick skin; thin skin contains dermal appendages. In general, the skin consists of three layers, the epidermis, dermis, and hypodermis. The epidermis is composed of five layers (stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, which is absent in thin skin type, and stratum corneum) from the innermost up to the superficial layer, respectively. The interdigitation of epidermis with the dermis forms epidermal papillae and dermal ridges throughout the basement membrane. While the dermis is divided into two layers of connective tissue, the upper papillary layer, and the deeper layer, which is the reticular layer that contains skin appendages (hair follicles, sebaceous gland, sweat gland, pili muscle, blood vessels, and sensory corpuscles). Finally, the hypodermis, which is the innermost layer beneath the dermis that is known as the subcutaneous fascia containing adipose tissue [25]

Wound healing stages

Wound healing represents a natural morphogenic response involving inflammatory cells, immune cells, and biochemical factors gathering for recovering the skin layers including cellular structure, restoring the anatomical shape and physiological function [26, 27].

Wound healing stages was demonstrated in Figure 1 as following: haemostasias, which occurs immediately after the injury, followed

by clot formation by triggering the release of platelet granules. Then the inflammation phase is triggered by inflammatory mediators, which recruit polymorphonuclear cells in the wound site. The proliferative phase occurs with the formation of blood vessels, production of fibroblasts and collagen deposition for granulation tissue development and finally, the remodeling phase in which granulation tissue maturation occurs and replaced by similar or close structure to the original tissue [26].

Growth factors have a critical role in improving the healing process. Epidermal growth factor (EGF) and hepatocyte growth factor (HGF) induce epithelial cells to grow, proliferate and migrate [28, 29]. While angiogenesis and vascularization processes are enhanced by a vascular endothelial growth factor (VEGF) and a platelet-derived growth factor (PDGF) [30]. The role of various growth factors included in wound healing mechanism was described in Table 1.

Growth factors and wound healing

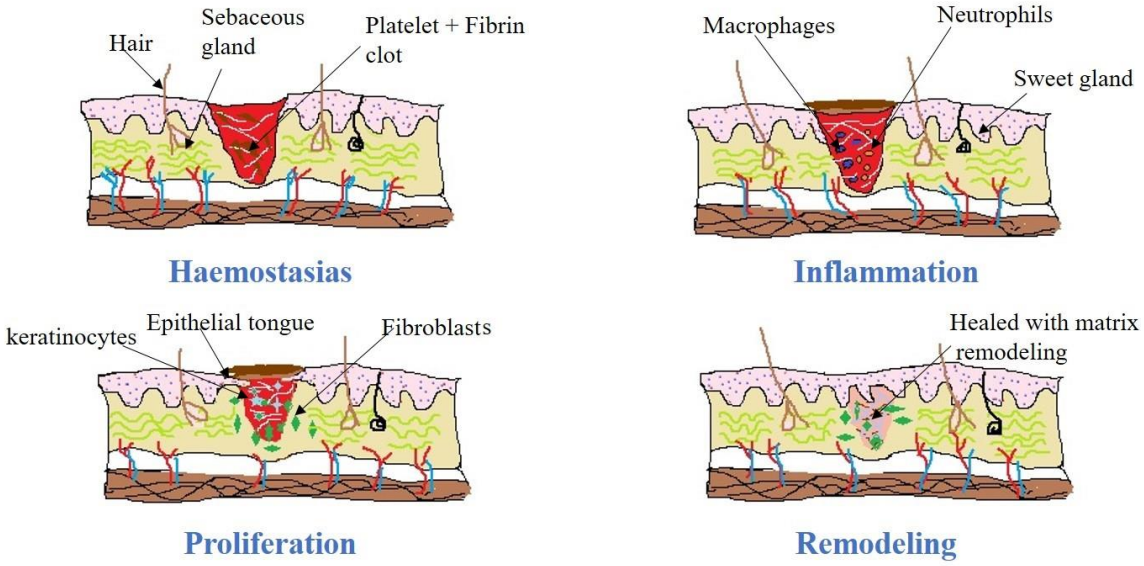

Fig. 1: Wound healing includes four stages, haemostasias, inflammation, proliferation and remodeling.

Table 1: Some important growth factors that play an essential role in wound healing.

Growth factor	Function
EGF	Stimulating fibroblasts proliferation, which responsible for producing collagen and accelerating wound healing [31, 32].
KGF	Inducing keratinocyte growth [33].
TGF-β1	Inducing migration and growth of fibroblasts and keratinocytes [34].
TGF-β2	Stimulating angiogenesis and vascularization [35].
HGF, TGF-β3	Enhancing healing progress and reducing scar formation [36, 37].
VEGF	Inducing angiogenesis [38].
PDGF	Directing fibroblasts and macrophages to the wounded region [39].
bFGF	Enhancing and accelerate wound healing, promoting collagen fibers deposition and extracellular matrix remodeling [40].

Growth factors participate in wound healing; EGF: epidermal growth factor, KGF: keratinocyte growth factor, TGF-β: beta-transforming growth factor, HGF: hepatocyte growth factor, VEGF: vascular endothelial growth factor, PDGF: platelet-derived growth factor and bFGF: basic fibroblast growth factor.

Types of wounds

Wound degree could be acute such as burn, abscess, unhealed surgical wound, open wound trauma, or could be chronic wounds such as diabetic ulcer, pressure ulcer, arterial leg ulcer, and venous leg ulcer [41, 42].

According to Jeschke *et al*. [43], dermal wounds can be classified based on the depth of the wound into superficial in epidermis layer, partially-thickness wounds up to the dermis, deep partially-thickness wounds, and full-thickness cutaneous wounds; the last type are characterized by the full damage of the epithelial layer. This type of wound heals by contracting wound edges and re-epithelialization, resulting in functional disorder and morphological distortion.

Acute wounds establish normal wound healing stages, ended with a satisfied and well-organized restoration of skin tissue layers [44]. In contrast, chronic wounds take a long time for healing and might lead to healing disorder [45]; therefore, the delayed healing makes open wounds to be exposed to microbial infection because the prolonged inflammatory phase leads to interrupting the healing process [46]. This results in a prolonged release of inflammatory growth factors and cytokines such as platelet-derived factors, transforming growth factor-beta (TGF-β), interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), which lead to an increase in proteases levels more than the inhibitors, which by the way lead to inflammation and proliferation delay hence scar formation [47].

The prolonged inflammatory stage in chronic wounds resulted in upregulation of the inflammatory cells, which in turn raised the reactive oxygen species (ROS) in the extracellular matrix (ECM) of the wounded tissue [45]. It was detected that when the wound is combined with reduced immunity, it may lead to bacterial infection, which affects the healing quality by causing scar formation and may be associated with severe pain. Hence, a need for a therapeutic scaffold with pain calming properties is desired [48].

It is necessary to modulate the environment during healing stages to remain natural healing process by controlling the interaction of inflammatory cells, the extracellular matrix, and the cytokines by applying dressings. This prevents infections and dehydration, which may be compatible with other topical therapeutic agents [22, 49], such biological materials must be non-toxic, non-immunogenic, or do not induce inflammation, and should be protectable against disease transmission [50].

Kaasi *et al*. [51] reported that the maximum wound size needed for spontaneous healing and well-organized tissue regeneration without special treatment is one cm in diameter, so it was claimed that the wound measuring two cm or more in diameter would need medical curing.

In veterinary medicine, the wound healing process is more complicated due to each species has its special characteristics; wounds in large animals such as horses, usually heal more slowly with the development of exuberant granulation tissue [52].

Stem cells as a promising technique for organs relief

Stem cells can be extracted from bone marrow stroma and other tissues such as umbilical cord blood, adipose tissue, with phenotypic heterogeneity [20, 21, 53].

Stem cells are characterized by self-renewal and the ability to differentiate into specific different types of cells, as the pluripotent stem cells are considered one of the chief classes of stem cells that can differentiate into any type of cells including embryonic cells. While the multipotent class of stem cells is restricted in differentiation into limited types of adult cells [54]. Multipotent stem cells exist in almost tissues and have the potency to differentiate into different types of cells [55].

It was found that umbilical cord blood is rich in hematopoietic stem cells (HSCs), which is characterized by less risk of human leukocyte antigen (HLA) matching since these cells can be cryopreserved for various future needs such as transplantation. This is due to special characteristics of umbilical cord blood (UCB) stem cells that reduce the risk of graft-versus-host-disease (GVHD) [56-59].

Mesenchymal stem cells (MSCs) are the common source of multipotent cells and can be derived from different tissues including

bone marrow, umbilical cord blood, Wharton's jelly, bone, adipose tissue, and peripheral blood. MSCs are characterized by plastic-adherent ability when maintained in tissue culture container, and are characterized by specific surface cell markers CD105, CD90, and CD73, while lacking surface markers of CD45, CD34, CD14, CD11b, CD19, CD79, and HLA-D. These cells are characterized by the ability to differentiate into mesodermal tissues such as adipose tissue, cartilage, bone, and muscular tissue [60- 61].

Nakagawa *et al*. [62] reported that MSCs could differentiate into neuronal-derived tissue of the ectodermal origin, which is known as the trans-differentiation phenomenon, where a cell from one germ layer (mesodermal) tends to differentiate into neuronal tissue (ectodermal).

Stem cells applications in wound healing

MSCs have received considerable attention for modulating wound repair in regenerative medicine; these cells were to be applied in tissue and organs restoration and curing of GVHD and autoimmune diseases due to their specific immune regulatory properties [63].

MSCs enhance the wound healing process, even more, chronic wounds resulting from diabetes mellitus, ischemia, or radiation exposure [64].

It was found that inflammation and oxidative stress generated during normal wound healing attract mesenchymal stem cells at the wound area and conduct to self-renewal; proliferation and also support wound healing through differentiation [65], re-epithelialization, and tissue granulation, and inducing vascularization [66]. It was found in a previous study that human umbilical cord blood (hUCB-MSCs) accelerated the healing of diabetic wounds by stimulating keratinocytes, fibroblast proliferation, and neovascularization [67].

Stem cells applications in wound healing in veterinary medicine

Spaas *et al.* [68] were the first who applied peripheral blood stem cells isolated from horse blood for treatment of adult horses with wounds induced from three months without any response to common therapies. After injection with stem cells, tissue regeneration was noticed within one month with the formation of small scars in the center of the wound and hair growth at the edges.

Also, it was reported that intradermal injection of allogeneic MSC in the canine model accelerated the closure of full-thickness cutaneous wounds with increased cellular proliferation, angiogenesis, and decrease inflammatory response [69]. In recent few years, a progress in stem cells applications in veterinary medicine for wound therapy in different large animals was noticed as summarized in Table 2.

Table 2: Applications of different sources of stem cells in wound healing in different experimental large animals.

Concept	Animal model
MSCs derived from fetal origin amniotic fluid and adult bone marrow for treatment full-thickness wound [70].	Capra hircus (goat)
MSCs isolated from peripheral blood applied for healing full-thickness wound [71].	Bergamasca sheep
ADSCs improved healing of full-thickness wound [72].	Yorkshire pigs
MSCs derived from bone marrow, attenuated hypertrophic scar formation in full-thickness wound in ear skin [73].	Rabbit
Allogenic MSCs extracted from adipose tissue for treatment of chronic cutaneous wound [74].	Canine (dog)
MSCs isolated from umbilical cord for treatment forelimb and thorax full-thickness wound [75].	Equine

MSCs: Mesenchymal stem cells; ADSCs: Adipose-derived stem cells.

Biological membranes, skin substitutes, and scaffolds

Biological membranes are biomaterials of a polymeric nature that cover wounds to assist in the acceleration of wound healing, which gives mechanically protection and functionally replaces the damaged skin permanently or temporarily [76].

Skin substitute biological materials are commonly identified by various terms, such as biological skin substitutes, skin substitute, bio-engineered skin, tissue-engineered skin, bio-constructs, living dermal replacements, artificial skin [77], and scaffolds; these are applied *in vivo* for tissue or organs regeneration. The polymeric type of the substitute or the scaffold can be natural, synthetic or semi-synthetic. The type and structural properties of the substitute such as biodegradation rate, permeability, and temporary mechanical support influence tissue regeneration rate by regulating cellular behavior [78].

Various techniques for scaffold manufacturing: classical methods and up-to-dates

Various wound dressings were developed based on synthetic or natural polymers such as chitosan, cellulose, and collagen [22, 23]. Here we are going to describe in brief some scaffolding techniques with different resulted specific characteristics.

a) Solvent casting or salt leaching

This method is considered as a classic technique for scaffolding. It depends on salt or progens dispersion into a polymer solution then left till solidification or cross-linked, and then desolating the salt or progen, resulting in a foam-like structure with irregular arrangement pores and irregular microstructure with pore size range from 50 to 600µm; the polymer must be non-toxic for cells [79].

b) Gas foaming

The gas foaming technique is established by adding a foaming agent as sodium bicarbonate, into the polymer solution, then processed under high pressure to release resulting gases and lyophilized [80]. This technique was previously applied with stem cells for bone regeneration with satisfying results [81].

c) Melt molding

This technique depends on mixing the polymer powder especially poly lactic glycolic acid (PLGA) with gelatin microspheres under heating conditions then the mold is placed in water for dissolving gelatin spheres. This resulted in a porous structure with irregular pores size according to the size of the microspheres, and the use of toxic solvent is prevented to avoid cells death [82].

d) Freeze-drying

The polymer solution was frozen then applied in freeze dryer to remove ice crystals through sublimation process under high pressure, leaving the porous spongy 3D structure; this was is called the lyophilization process. This method was applied in several studies due to the ability to control porosity by controlling the freezing temperature and polymer concentration [83, 84].

e) Electrospinning

The concept of this technique depends on the application of electrostatic power (10-40kV) to the polymer solution in a syringe-like instrument with a nozzle for forcing the ionized polymer solution to pass through the nozzle. Thereafter, it is precipitated on an aluminum membrane to form a 3D woven fibers structure in nanometric scale or micrometric scale according to the voltage used and polymer concentration [85, 86].

f) Three-dimensional printing technology

Three-dimensional printing technology was developed for scaffolding by different techniques, such as stereolithography and rapid prototyping where their scientific concept applied on the deposition of a thin layer on the polymer over each other than solidified by the ultraviolet [87, 88]**.** This technique was modified in a particular selective laser sintering to manufacture a 3D structure by application of a powerful laser to polymer powder [87]. 3D printing is the latest technology in tissue engineering, using a modified inkjet printer for regeneration of the full-thickness skin in mice tissue by applying fibroblasts and keratinocytes in the inkjet

printer [89] and also stem cells derived from amniotic fluid with the aid of specific software [90].

Tissue-engineered skin application for wound healing

A combination of MSCs with biodegradable scaffolds or membranes resulted in a progressive decrease in wound size, highly increased vascularity, and dermal thickness in chronic ulcers [91].

The biomaterial for skin regeneration should be biodegradable, with similar physical and mechanical properties, and supportable for normal tissue reconstruction and should be analgesic, antimicrobial, and prevent dehydration of tissue fluid, and heat regulation in the wound region. It is important to possess a long shelf life, cost-effective, the mechanical stability of scaffold structure is important for maintaining cellular proliferation and differentiation [92, 93].

Several previous studies demonstrated different types of scaffolds and cells for wound healing as shown in Figure 2. It was found that nanofiber scaffold cultured with UCB-MSCs in mice facilitated wound healing ability [94].

A naturally derived chitosan bilayer porous biopolymer scaffold has been developed for skin tissue engineering with pore sizes ranging from 50 to 150 μm. It was biocompatible, as it induced neither cytotoxicity nor irritation [95].

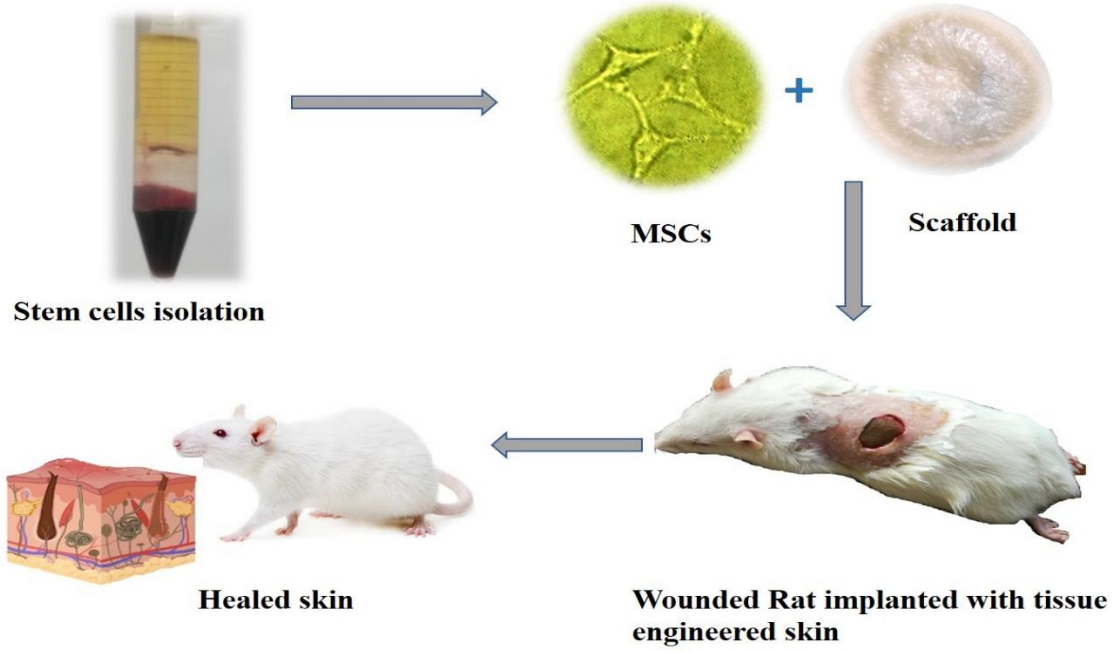

Figure 2: Skin engineering required several steps, the isolated cells cultured in the scaffold then implanted in the wounded region for accelerating wound healing and mimicking natural skin regeneration.

Skin engineering applications in veterinary medicine

In the veterinary field, biological membranes showed an important technical feature, as they can be used in non-clinical conditions [96].

The application of collagen bio-membrane was reported in groups of skin wounded cats and dogs with different causes for skin injury, such as burns, surgical excisions, and traumas. The wounds were of second or third-degree burns, which are extending into the dermis or subcutaneous layers of the skin. The treatment with collagen bio-membrane resulted in acceleration of wound healing about three times more than the non-treated wound [51]. In addition, Kawamoto *et al*. [97] fabricated a novel three-dimensional scaffold of collagen and graphene oxide for wound healing in the dogs.

Chitosan and chitosan derivatives applications

Chitosan is a polysaccharide glucose amine polymer derived from the alkaline deacetylation of chitin and this is the second most abundant biopolymer after cellulose [98, 99]. Crustaceans are considered as the main sources of chitosan then insects, and the cell wall of fungi [24]. Chitosan has a good solubilization rate, which allows for producing various pharmaceutical formulations such as films, powders, hydrogels, pastes, or membranes [100, 101]. Chitosan was extracted from two different sources, one from chitin from shrimp shells, and the other was from a Desert Locust. The study observed higher efficiency in healing for chitosan extracted from Desert Locust in contrast, minimal healing was observed in the control group in the same time [24].

Chitosan is considered an inexpensive product; it has good antimicrobial, anti-inflammatory, analgesic, and healing activities, especially in cutaneous wounds [99, 101]. Chitosan has an analgesic effect when applied to an open wound by blocking the nerve endings and providing a calming effect. In addition, chitosan triggers clotting by binding to erythrocytes, stimulating the secretion of platelet-derived growth factor (PDGF) and transforming growth factor (TGF) [102].

Chitosan approaches in wound healing

Several previous studies documented that chitosan prevented wound infection during wound repair, as well as many other advantages such as an analgesic effect and hemostatic activity [100]. Mezzana *et al.* [103] studied the cytotoxicity of chitosan hydrogel and found that the by-products yielded after degradation are non-cytotoxic and enhance cellular adhesion and proliferation. In addition, chitosan could be manufactured in different forms for wound healing. It could be used as scaffolds, nanofibers, filaments, membranes, powders, gels, granules, sponges, or as a composite. Moreover, chitosan is characterized by activation of the polymorphonuclear cell (PMN) and fibroblasts, enhance the migration of giant cells, production of cytokines, and simulation of collagen IV synthesis, which led to granulation tissue organization and reducing in the number of inflammatory cells especially in the chronic wound regions [103]. Chitosan with a high degree of deacetylation (DDA) of ≥ 89% strongly stimulating fibroblasts proliferation more than chitosan with lower DDA, as chitosan induces early collagen deposition in wounds induced in rats [104].

Histological examination confirmed that the epithelialization rate was increased and the deposition of collagen in the dermis was well organized by covering the wounds with chitosan [105]. Various animal studies on using chitosan to treat or prevent different types of wound infection had been carried out. Jayakumar *et al.* [16] detected that chitosan rapidly killed the microbial cells in wounds and reduced the mortality of the animals in cases of severe infections. Moreover, chitosan sponge was preferred to be applied for wound healing because they can absorb exudates resulting from the wound and promoting tissue regeneration since the structure of spongy scaffold with open interconnected micro-pores can absorb high amounts of fluids more than twenty folds, which offer well cell interaction, and keep scaffold flexibility [16].

Future directions in MSCs therapies

In recent previous few years, the future research direction reported several studies held and indicated a new concept which illustrating MSCs therapeutic mechanism depending on the MSCs extracellular vesicles (exosomes) throughout local paracrine that trigger migration and direction of MSCs into the injured tissues [106, 107]. Chuo *et al*. [108] reported that exosomes derived from MSCs release specific regenerative factors, which induce cell proliferation, stimuli immune responses, and promote angiogenesis. The direction of current research towards investigations of MSCs derived exosomes in different therapeutic aspects, due to the cell senescence during *in vitro* preparations and storage limitations leading to cellular loss and decreasing viability, which considered as a critical clinical challenge for therapeutic applications of MSCs [109].

Conclusion

Since the umbilical cord is considered as medical waste, as well as crustaceans shells

results in a huge amount of waste. Science investigated those waste products as an applicable source for which considered a jump in regenerative medicine. This is because MSCs could be cultured in living body, differentiate spontaneously into the targeted cells, and regenerate the damaged tissue without rejection complications. Hence, MSCs is considered as a safe way for damaged tissue treatment, while chitosan is a versatile and multi-applicable natural product in the medical field, agriculture, and industry. The combination between chitosan, which is a waste product from crustacean shells, and MSCs extracted from umbilical cord blood resemble a low cost and very available source for tissue engineering applications by the manufacturing of artificial organs to be applied in therapies of organs transplantation fields, especially in our research in treatment of wound healing complications.

Reference

[1] Venkatesan, J., Vinodhini, P. A., Sudha, P. N and Kim, S. K. (2014): Chitin and chitosan composites for bone tissue regeneration. Adv Food Nutr Res, 73, 59–81.

[2] Laurence, J., Baptista, M. P and Atala, A. Eds. (2015): Translating regenerative medicine to the clinic., Academic Press, 354.

[3] Ikada, Y. (2006): Challenges in tissue engineering. J R Soc Interface, 3(10): 589–601.

[4] Bolognin, S.; Fossépré, M.; Qing, X.; Jarazo, J.; Ščančar, J.; Moreno, E. L. Nickels, S. L., Wasner, K., Ouzren, N., Walter, J., Grünewald, A., Glaab, E., Salamanca, L., Fleming, R., Antony, P., and Schwamborn, J. C. (2019): 3D cultures of Parkinson's disease-specific dopaminergic neurons for high content phenotyping and drug testing. Adv Sci, 6(1): 1800927.

[5] Esmaeili, A. and Haseli, M. (2017): Optimization, synthesis, and characterization of coaxial electrospun sodium carboxymethyl cellulose-graft-methyl acrylate/poly (ethylene oxide) nanofibers for potential drug-delivery applications. Carbohydr Polym, 173: 645–653.

[6] Oh, B. H.; Bismarck, A. and Chan-Park, M. B. (2014): High internal phase emulsion templating with self-emulsifying and thermo responsive chitosan-graft-PNIPAM-graft-oligoproline. Biomacromolecules, 15(5): 1777–1787.

[7] Liang, Y.; Zhao, X.; Ma, P. X.; Guo, B.; Du, Y. and Han, X. (2019): pH-responsive injectable hydrogels with mucosal adhesiveness based on chitosan-grafted-dihydrocaffeic acid and oxidized pullulan for localized drug delivery. J. Colloid Interface Sci, 536: 224–234.

[8] Gautam, S.; Chou, C. F.; Dinda, A. K.; Potdar, P. D. and Mishra, N. C. (2014): Surface modification of nanofibrous polycaprolactone/gelatin composite scaffold by collagen type I grafting for skin tissue engineering. Mater Sci Eng C Mater Biol Appl, 34: 402–409.

[9] Ospina-Orejarena, A.; Vera-Graziano, R.; Castillo-Ortega, M. M.; Hinestroza, J. P. and Rodriguez-Gonzalez, M.; Palomares-Aguilera, L.; Morales-Moctezuma, M. and Maciel-Cerda, A. (2016): Grafting collagen on poly (lactic acid) by a simple route to produce electrospun scaffolds, and their cell adhesion evaluation. J Tissue Eng Regen Med, 13: 375–387.

[10] Müller, F. A.; Müller, L.; Hofmann, I.; Greil, P.; Wenzel, M. M. and Staudenmaier, R. (2006): Cellulose-based scaffold materials for cartilage tissue engineering. Biomaterials, 27(21): 3955–3963.

[11] Kumar, K. M. N. V. R (2017): Handbook of Polyester Drug Delivery Systems, Pan Stanford, 595e649.

[12] Wubneh, A.; Tsekoura, E. K.; Ayranci, C. and Uludağ, H. (2018): Current state of fabrication technologies and materials for bone tissue engineering. Acta biomate, 80: 1–30.

[13] Majtán, J.; Bíliková, K.; Markovič, O.; Gróf, J.; Kogan, G. and Šimúth, J. (2007): Isolation and characterization of chitin from bumblebee (Bombus terrestris). Int J Biol Macromol, 40(3): 237-241.

[14] Cardea, S.; Pisanti, P. and Reverchon, E. (2010): Generation of chitosan

nanoporous structures for tissue engineering applications using a supercritical fluid assisted process. J Supercrit Fluids, 54(3): 290-295.

[15] Sánchez-Duarte, R. G.; Sánchez-Machado, D. I.; López-Cervantes, J. and Correa-Murrieta, M. A. (2012): Adsorption of allura red dye by cross-linked chitosan from shrimp waste. Water Sci Technol, 65(4): 618-623.

[16] Jayakumar, R.; Prabaharan, M.; Sudheesh Kumar, P.T.; Nair, S.V. and Tamura, H. (2011): Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv, 29(3): 322–337.

[17] Forraz, N. and McGuckin, C.P. (2011): The umbilical cord: A rich and ethical stem cell source to advance regenerative medicine. Cell proliferation, 44(Suppl 1): 60-69.

[18] Agius, C. and Blundell, R. (2012): The cutting edge in stem cell medical applications. Res J Med Sci, 2: 47-50.

[19] Mckenna, D.H.; Kadidlo, D.M.; Mccullough, J. and Regan, D.M. (2011): Umbilical cord blood. In: Roback, J.D., Grossman, B.J., Harris, T. and Hillyer, C.D., Eds., Technical manual. American association of blood banks, Bethesda, 823-847.

[20] Pace, P. and Blundell, R. (2016): Stem Cells: Daddy or Chips? —An Up-to-Date Review on Ground-Breaking Discoveries in Stem Cell Research with Special Attention to iPSC Applications in Osteoarthritis. Stem Cell Discovery, 6(1): 39-44.

[21] Velnar, T.; Bailey, T. and Smrkolj, V. (2009): The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res, 37(5): 1528-1542.

[22] Ma, Y.; Xin, L.; Tan, H.; Fan, M.; Li, J.; Jia, Y., Ling, Z., Chen, Y. and Hu, X. (2017): Chitosan membrane dressings toughened by glycerol to load antibacterial drugs for wound healing. Mater Sci Eng C Mater Biol Appl ,81: 522-531.

[23] Morgado, P.I.; Lisboa, P.F.; Ribeiro, M.P.; Miguel, S.P.; Simões, P.C.; Correia, I.J. and Aguiar-Ricardo, A. (2014): Poly (vinyl alcohol)/chitosana symmetrical membranes: Highly controlled morphology toward the ideal wound dressing.
J Membr Sci, 69: 262-271.

[24] Marei, N.H.; El-Mazny, W.; El-Shaer, A.; Zaki, K.D.; Hussein, Z.S. and AbdEl-Samie, E.M. (2017): Enhanced wound healing activity of desert locust (*Schistocerca gregaria*) vs. shrimp (*Penaeus monodon*) chitosan-based scaffolds. Int J Biol Macromol, 97: 23-33.

[25] Yousef, H., Alhajj, M., Sharma, S. (2021): Anatomy, Skin (Integument), Epidermis. In: StatPearls. Treasure Island (FL): StatPearls Publishing.

[26] Andrade, T.A.M.; Iyer, A.; Das, P.K.; Foss, N.T.; Garcia, S.B.; Coutinho-N etto, J.; Frade, M.A.C. (2011): The inflammatory stimulus of a natural latex biomembrane improves healing in mice. Braz J Med Biol Res, 44(10):1036-1047.

[27] Vyas, K. S. and Vasconez, H.C. (2014): Wound healing: biologics, skin substitutes, biomembranes and scaffolds. Healthcare (Basel), 2(3): 356-400.

[28] Lee, J. H.; Bae, I. H.; Choi, J. K.; Park, J. W. (2013): Evaluation of a highly skin permeable low molecular-weight protamine conjugated epidermal growth factor for novel burn wound healing therapy. J Pharm Sci, 102(11): 4109-4120.

[29] Yoshida, S.; Yamaguchi, Y.; Itami, S.; Yoshikawa, K.; Tabata, Y.; Matsumoto K. and Nakamura, T. (2003): Neutralization of hepatocyte growth factor leads to retarded cutaneous wound healing associated with decreased neovascularization and granulation tissue formation. J Invest Dermatol, 120(2): 335-343.

[30] Demidova-Rice, T. N.; Hamblin, M. R. and Herman, I. M. (2012): Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 2: role of growth factors in normal and pathological wound healing: therapeutic potential and methods of delivery. Adv Ski Wound Care, 25(8): 349-370.

[31] Kim, H.; Kong, W. H.; Seong, K. Y.; Sung, D. K.; Jeong, H.; Kim, J. K.; Yang, S. Y. and Hahn, S. K. (2016): Hyaluronate-epidermal growth factor conjugate for skin wound healing and

regeneration. Biomacromolecules, 17(11): 3694-3705.

[32] Gainza, G.; Bonafonte, D. C.; Moreno, B.; Aguirre, J. J.; Gutierrez, F. B.; Villullas, S.; Pedraz, J. L.; Igartua, M. and Hernandez, R. M. (2015): The topical administration of rhEGF-loaded nanostructured lipid carriers (rhEGF-NLC) improves healing in a porcine full-thickness excisional wound model. J Control Release, 197: 41-47.

[33] Geer, D. J.; Swartz, D. D. and Andreadis, S. T. (2005): Biomimetic delivery of keratinocyte growth factor upon cellular demand for accelerated wound healing *in vitro* and *in vivo*. Am J Pathol. 167(6): 1575–1586.

[34] Puolakkainen, P. A.; Twardzik, D. R.; Ranchalis, J. E.; Pankey, S. C.; Reed, M. J. and Gombotz, W. R. (1995): The enhancement in wound healing by transforming growth factor-beta 1 (TGF-beta 1) depends on the topical delivery system. J Surg Res, 58: 321–329.

[35] Kojima, K.; Ignotz, R. A.; Kushibiki, T.; Tinsley, K. W.; Tabata, Y. and Vacanti, C. A. (2004): Tissue-engineered trachea from sheep marrow stromal cells with transforming growth factor β2 released from biodegradable microspheres in a nude rat recipient. J Thorac Cardiovasc Surg, 128(1): 147-153.

[36] Xiao, Z. and Xi, C. (2013): Hepatocyte growth factor reduces hypertrophy of skin scar: *in vivo* study. Adv Ski Wound Care, 26(6): 266-270.

[37] Li, M.; Qiu, L.; Hu, W.; Deng, X.; Xu, H.; Cao, Y.; Xiao, Z.; Peng, L.; Johnson, S.; Alexey, L.; Kingston, P. A.; Li, Q. and Zhang, Y. (2018): Genetically-modified bone mesenchymal stem cells with TGF-β3 improve wound healing and reduce scar tissue formation in a rabbit model. Exp Cell Res, 367: 24-29.

[38] Elçin, Y. M.; Dixit, V. and Gitnick, G. (2001): Extensive *in vivo* angiogenesis following controlled release of human vascular endothelial cell growth factor: implications for tissue engineering and wound healing. Artif Organs, 25(7): 558–565.

[39] Wieman, T. J.; Smiell, J. M. and Su, Y. (1998): Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. Diabetes care, 21(5): 822-827.

[40] Yang, Y.; Xia, T.; Zhi, W.; Wei, L.; Weng, J.; Zhang, C. and Li, X. (2011): Promotion of skin regeneration in diabetic rats by electrospun core-sheath fibers loaded with basic fibroblast growth factor. Biomaterials, 32(18): 4243-4254.

[41] Guest, J. F.; Ayoub, N.; McIlwraith, T.; Uchegbu, I.; Gerrish, A.; Weidlich, D.; Vowden, K. and Vowden, P. (2015): Health economic burden that wounds impose on the National Health Service in the UK. BMJ Open, 5(12): e009283.

[42] Guest, J. F.; Ayoub, N.; McIlwraith, T.; Uchegbu, I.; Gerrish, A.; Weidlich, D.; Vowden, K. and Vowden, P. (2017): Health economic burden that different wound types impose on the UK's National Health Service. Int Wound J, 14(2): 322-330.

[43] Jeschke, M. G.; Gauglitz, G. G.; Kulp, G. A.; Finnerty, C. C.; Williams, F. N.; Kraft, R.; Suman, O. E.; Mlcak, R. P. and Herndon, D. N. (2011): Long-term persistance of the pathophysiologic response to severe burn injury. PloS one, 6(7): e21245.

[44] Lindholm, C. and Searle, R. (2016): Wound management for the 21st century: Combining effectiveness and efficiency. Int Wound J, 13(Suppl 2): 5-15.

[45] Ben-Porath, I. and Weinberg, R.A. (2005): The signals and pathways activating cellular senescence. Int J Biochem Cell Biol, 37(5): 961-976.

[46] James, G. A.; Swogger, E.; Wolcott, R.; Pulcini, E. D.; Secor, P.; Sestrich, J.; Costerton, J. W. and Stewart, P. S. (2008): Biofilms in chronic wounds. Wound Repair Regen, 16(1): 37-44.

[47] McCarty, S. M. and Percival, S. L. (2013): Proteases and Delayed Wound Healing. Adv Wound Care (New Rochelle), 2(8): 438-447.

[48] Kim, J. Y.; Song, S. H.; Kim, K. L., Ko, J. J.,Jm, J. E., Yie, S. W., Ahn, Y. K., Kim, D. K. and Suh, W. (2010): Human cord blood derived endothelial progenitor cells and

their conditioned media exhibit therapeutic equivalence for diabetic wound healing. Cell Transplantation, 19(12):1635-1644.

[49] Bankoti, K.; Rameshbabu, A. P.; Datta, S.; Maity, P. P.; Goswami, P.; Datta, P. and Dhara, S. (2017): Accelerated healing of full-thickness dermal wounds by macroporous waterborne polyurethane-chitosan hydrogel scaffolds. Mater Sci Eng C Mater Biol Appl, 81: 133-143.

[50] Bano, I.; Arshad, M.; Yasin, T.; Ghauri, M.A. and Younus, M. (2017): Chitosan: A potential biopolymer for wound management. Int J Biol Macromol, 102: 380-383.

[51] Kaasi, A.; Lima-Neto, J. F.; Matiello-Filho, J. A.; Calejo, M. H.; Jardini, A. L.; and Kharmandayan, P. (2018): Regenerative collagen biomembrane: Interim results of a phase I veterinary clinical trial for skin repair. F1000Research, 7: 729.

[52] Theoret, C. L. and Wilmink, J. M. (2013): Aberrant wound healing in the horse: naturally occurring conditions reminiscent of those observed in man. Wound Repair Regen. *21*(3): 365–371.

[53] Bourin, P.; Bunnell, B. A.; Casteilla, L.; Dominici, M.; Katz, A. J.; March, K. L.; Redl, H.; Rubin, J. P.; Yoshimura, K. and Gimble, J. M. (2013): Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy, 15(6): 641–648.

[54] Biehl, J. K. and Russell, B. (2009): Introduction to stem cell therapy. J Cardiovasc Nurs, 24(2): 98-103.

[55] Ratajczak, M. Z.; Zuba-Surma, E.; Kucia, M.; Po-niewierska, A.; Suszynska, M. and Ratajczak, J. (2012): Pluripotent and multipotent stem cells in adult tissues. Adv Med Sci, 57(1): 1-17.

[56] Smith, A. R. and Wagner, J. E. (2009): Alternative haematopoietic stem cell sources for transplantation: place of umbilical cord blood. Br J Haematol, 147(2): 246-261.

[57] Henig, I. and Zuckerman, T. (2014): Hematopoietic stem cell transplantation-50 years of evolution and future perspectives. Rambam Maimonides Med J, 5(4): e0028

[58] Matsumoto, M. M. and Matthews, K. R. W. (2015): A need for renewed and cohesive US policy on cord blood banking. Stem Cell Rev Rep,11(6): 789-797.

[59] Tiercy, J. M. (2016): How to select the best available related or unrelated donor of hematopoietic stem cells? Haematologica, 101(6): 680-687.

[60] Augello, A.; Kurth, T.B. and De, B.C. (2010): Mesenchymal stem cells: a perspective from *in vitro* cultures to in vivo migration and niches. Eur Cell Mater, 20: 121-133.

[61] Barzilay, R.; Melamed, E. and Offen, D. (2009): Introducing transcription factors to multipotent mesenchymal stem cells: making transdifferen-tiation possible. Stem Cells, 27(10): 2509-2515.

[62] Nakagawa, H.; Akita, S.; Fukui, M.; Fujii, T. and Akino, K. (2005): Human mesenchymal stem cells successfully improve skin substitute wound healing, Br J Dermatol, 153(1): 29-36.

[63] Bajada, S.; Mazakova, I.; Richardson, J. B. and Ashammakhi, N. (2008): Updates on stem cells and their applications in regenerative medicine. J Tissue Eng Regener Med, 2(4): 169-183.

[64] Le Blanc, K. and Mougiakakos, D. (2012): Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immuno, 12(5): 383–396.

[65] Liu, L.; Yu, Y.; Hou, Y.; Chai, J.; Duan, H.; Chu, W.; Zhang, H.; Hu, Q. and Du, J. (2014): Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PloS one, 9(2): e88348.

[66] Seifert, A. W.; Monaghan, J. R.; Voss, S. R. and Maden, M. (2012): Skin regeneration in adult axolotls: a blueprint for scar-free healing in vertebrates. PloS one, 7(4): e32875.

[67] Kanji, S.; Das, M., Aggarwal, R., lu, J., Joseph, M., Basu, S., Pompili, V. J and Das, H. (2014): Nanofiber-expanded

human umbilical cord blood-derived CD34+ cell therapy accelerates murine cutaneous wound closure by attenuating pro-inflammatory factors and secreting IL-10. Stem Cell Res, 12(1): 275-288.

[68] Spaas, J. H.; Broeckx, S.; Van de Walle, G. R. and Polettini, M. (2013): The effects of equine peripheral blood stem cells on cutaneous wound healing: a clinical evaluation in four horses. Clin Exp Dermatol, 38(3): 280-284.

[69] Kim, J. W.; Lee, J. H.; Lyoo, Y. S.; Jung, D. I. and Park, H. M. (2013): The effects of topical mesenchymal stem cell transplantation in canine experimental cutaneous wounds. Vet Dermatol, 24(2): 242-253.

[70] Pratheesh, M. D., Dubey, P. K., Gade, N. E., Nath, A., Sivanarayanan, T. B., Madhu, D. N., Somal, A., Baiju, I., Sreekumar, T. R., Gleeja, V. L., Bhatt, I. A., Chandra, V., Amarpal, Sharma, B., Saikumar, G., and Taru Sharma, G. (2017): Comparative study on characterization and wound healing potential of goat (*Capra hircus*) mesenchymal stem cells derived from fetal origin amniotic fluid and adult bone marrow. Vet Sci Res J, 112: 81-88.

[71] Martinello, T., Gomiero, C., Perazzi, A., Iacopetti, I., Gemignani, F., DeBenedictis, G.M., Ferro, S., Zuin, M., Martines, E., Brun, P., Maccatrozzo, L., Chiers, K., Spaas, J.H., Patruno, M. (2018): Allogeneic mesenchymal stem cells improve the wound healing process of sheep skin. BMC Vet Res, 14: 202.

[72] James, I., Bourne, D., Silva, M., Havis, E., Albright, K., Zhang, L., Kostereva, N., Wang, S., DiBernardo, G., Guest, R., Lei, J., Almadori, A., Satish, L., Marra, K., Rubin, J.P. (2018): Adipose stem cells enhance excisional wound healing in a porcine model. J Surg Res, 229: 243-253.

[73] Hu, C. H., Tseng, Y. W., Chiou, C. Y., Lan, K. C., Chou, C. H., Tai, C. S., Huang, H. D., Hu, C. W., Liao, K. H., Chuang, S. S., Yang, J. Y., & Lee, O. K. (2019). Bone marrow concentrate-induced mesenchymal stem cell conditioned medium facilitates wound healing and prevents hypertrophic scar formation in a rabbit ear model. Stem Cell Res Ther, 10: 275.

[74] Enciso, N., Avedillo, L., Fermín, M. L., Fragío, C and Tejero, C. (2020): Cutaneous wound healing: canine allogeneic ASC therapy. Stem Cell Res Ther, 11: 1-14.

[75] Mund, S.J. K., Kawamura, E., Awang-Junaidi, A.H., Campbell, J., Wobeser, B., MacPhee, D.J., Honaramooz, A., Barber, S. (2020): Homing and engraftment of intravenously administered equine cord blood-derived multipotent mesenchymal stromal cells to surgically created cutaneous wound in horses: a pilot project. Cells, 9: 1162.

[76] Ha, T. L. B.; Quan, T.M., Vu, D.N, Si, DM. (2013): Naturally derived biomaterials: preparation and application. In Regenerative medicine and tissue engineering. In tech open, Chapter 11.

[77] Kim P. J.; Dybowski K. S. and Steinberg J. S. (2006): Feature: a closer look at bioengineered alternative tissues. Podiatry Today, 19: 38–55.

[78] Shevchenko, R. V.; James, S. L.; James, S. E. (2010): A review of tissue-engineered skin bio constructs available for skin reconstruction. J R Soc Interface, 7(43): 229-258.

[79] El-Kady, A. M.; Rizk, R. A.; Abd El-Hady, B. M.; Shafaa, M. W. and Ahmed, M. M. (2012): Characterization, and antibacterial properties of novel silver releasing nanocomposite scaffolds fabricated by the gas foaming/salt-leaching technique. J Genet Eng Biotechnol, 10: 229-238.

[80] Preethi Soundarya, S.; Haritha Menon, A.; Viji Chandran, S.; and Selvamurugan, N. (2018): Bone tissue engineering: Scaffold preparation using chitosan and other biomaterials with different design and fabrication techniques. Int J Biol Macromol, 119: 1228–1239.

[81] Ceccaldi, C.; Bushkalova, R.; Cussac, D.; Duployer, B.; Tenailleau, C.; Bourin, P.; Parini, A.; Sallerin, B. and Girod Fullana, S. (2017): Elaboration and evaluation of alginate foam scaffolds for soft tissue engineering. Int J Pharm, 524: 433–442.

[82] Mao, D.; Li, Q.; Li, D.; Tan, Y. and Che, Q. (2018): 3D porous poly (ε-caprolactone)/58S bioactive glass–sodium alginate/gelatin hybrid scaffolds prepared by a modified melt molding method for

bone tissue engineering. Mater. Des, 160: 1-8.

[83] Fellows, P. J. (2009): Food processing technology: principles and practice. 3rd ed. CRC press (Washington): Woodhead Publishing.

[84] Maji, K. and Dasgupta, S. (2017): Effect of β tricalcium phosphate nanoparticles additions on the properties of gelatin–chitosan scaffolds. Bioceram Dev Appl, 7: 2.

[85] Sill, T. J. and von Recum, H. A. (2008): Electrospinning: applications in drug delivery and tissue engineering. Biomaterials, 29(13): 1989-2006.

[86] Joseph, B.; Augustine, R.; Kalarikkal, N.; Thomas, S.; Seantier, B.; Grohens, Y. (2019): Recent advances in electrospun polycaprolactone based scaffolds for wound healing and skin bioengineering applications. Mater Today Commun, 19: 319-335.

[87] Gross, B. C.; Erkal, J. L.; Lockwood, S. Y.; Chen, C. and Spence, D. M. (2014): Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. Anal Chem, 86: 3240-3253.

[88] Peltola, S. M.; Melchels, F. P.; Grijpma, D. W. and Kellomäki, M. (2008): A review of rapid prototyping techniques for tissue engineering purposes. Ann Med, 40(4): 268-280.

[89] Binder, K. W.; Zhao, W.; Aboushwareb, T.; Dice, D.; Atala, A. and Yoo, J. J. (2010): In situ bioprinting of the skin for burns. J Am Coll Surg, 211: S76.

[90] Skardal, A.; Mack, D.; Kapetanovic, E.; Atala, A.; Jackson, J. D.; Yoo, J.; Soker, S. (2012): Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med, 1(11): 792–802.

[91] Wang, X.; Li, Q.; Hu, X.; Ma, L.; You, C.; Zheng, Y.; Sun, H.; Han, C. and Gao, C. (2012): Fabrication and characterization of poly(L-lactide-co-glycolide) knitted mesh-reinforced collagen-chitosan hybrid scaffolds for dermal tissue engineering. J Mech Behav Biomed Mater, 8: 204-215.

[92] Haastert-Talini, K., Geuna, S., Dahlin, L. B., Meyer, C., Stenberg, L., Freier, T., Heimann, C., Barwig, C., Pinto, L. F., Raimondo, S., Gambarotta, G., Samy, S. R., Sousa, N., Salgado, A. J., Ratzka, A., Wrobel, S. and Grothe, C. (2013): Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. *Biomaterials*, *34*: 9886–9904.

[93] Zhou, M., Qiao, W., Liu, Z., Shang, T., Qiao, T., Mao, C. and Liu, C. (2014): Development and *in vivo* evaluation of small-diameter vascular grafts engineered by outgrowth endothelial cells and electrospun chitosan/poly(ε-caprolactone) nanofibrous scaffolds. Tissue Eng Part A, 20(1-2): 79-91.

[94] Kanji, S., Das, M., Aggarwal, R., Lu, J., Joseph, M., Pompili, V. J. and Das, H. (2014): Nanofiber-expanded human umbilical cord blood-derived CD34+ cell therapy accelerates cutaneous wound closure in NOD/SCID mice, J Cell Mol Med, 18(4): 685-697.

[95] Lim, C. K.; Halim, A. S.; Zainol, I. and Noorsal, K. (2011): *In vitro* evaluation of a biomedical-grade bilayer chitosan porous skin regenerating template as a potential dermal scaffold in skin tissue engineering. Int J Polym Sci, 2011: 7.

[96] Drewnowska, O.; Turek, B.; Carstanjen, B. and Gajewski, Z. (2013): Chitosan a promising biomaterial in veterinary medicine. Pol J Vet Sci, 16(4): 843-848.

[97] Kawamoto, K.; Miyaji, H.; Nishida, E.; Miyata, S.; Kato, A.; Tateyama, A and Sugaya, T. (2018): Characterization and evaluation of graphene oxide scaffold for periodontal wound healing of class II furcation defects in dog. Int J Nanomed, 13: 2365.

[98] Behera, S. S.; Das, U.; Kumar, A.; Bissoyi, A. and Singh, A.K. (2017): Chitosan/TiO2 composite membrane improves proliferation and survival of L929 fibroblast cells: Application in wound dressing and skin regeneration. Int J Biol Macromol, 98: 329-340.

[99] Muxika, A.; Etxabide, A.; Uranga, J.; Guerrero, P. and De La Caba, K. (2017): Chitosan as a bioactive polymer: Processing, properties and applications. Int J Biol Macromol, 105(Pt2): 1358-1368.

[100] Gutha, Y., Pathak, J. L., Zhang, W., Zhang, Y. and Jiao, X. (2017):

Antibacterial and wound healing properties of chitosan/poly (vinyl alcohol)/zinc oxide beads (CS/PVA/ZnO). Int J Biol Macromol, 103: 234-241.

[101] Oryan, A. and Sahvieh, S. (2017): Effectiveness of chitosan scaffold in skin, bone and cartilage healing. Int J Biol Macromol, 104:1003-1011.

[102] Ribeiro, M.P.; Espiga, A.; Silva, D.; Baptista, P.; Henriques, J.; Ferreira, C.; Silva, J. C.; Borges, J. P.; Pires, E.; Chaves, P. and Correia, I. J. (2009): Development of a new chitosan hydrogel for wound dressing. Wound Repair Regen, 17(6): 817-824.

[103] Mezzana, P. (2008): Clinical efficacy of a new chitin nanofibrils-based gel in wound healing. Acta Chir Plast, 50(3): 81-84.

[104] Baxter, R. M.; Dai, T.; Kimball, J.; Wang, E.; Hamblin, M. R.; Wiesmann, W. P.; McCarthy, S. J. and Baker, S. M. (2013): Chitosan dressing promotes healing in third degree burns in mice: gene expression analysis shows biphasic effects for rapid tissue regeneration and decreased fibrotic signaling. J Biomed Mater Res Part A, 101(2): 340-348.

[105] Ong, S.Y.; Wu, J. and Moochhala, S. M. (2008): Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials, 29: 4323-4332.

[106] El-Naseery, N. I., Elewa, Y., Arafa, M., Sabbah, W. S. and Dessouky, A. A. (2021): Mesenchymal stem cells enhance AQP1 expression in the sublingual salivary gland of ovariectomized menopausal rat model. Ann Anat, 236: 151714.

[107] Abolgheit, S., Abdelkader, S., Aboushelib, M., Omar, E., Mehanna, R. (2021): Bone marrow-derived mesenchymal stem cells and extracellular vesicles enriched collagen chitosan scaffold in skin wound healing (a rat model). J Biomater Appl; 36(1):128-139.

[108] Chuo ST, Chien JC and Lai CP. (2018): Imaging extracellular vesicles: current and emerging methods. J Biomed Sci; 25: 91-12.

[109] Tang, Y., Zhou, Y., and Li, H. J. (2021): Advances in mesenchymal stem cell exosomes: a review. Stem Cell Res Ther, 12: 1-12.

الملخص العربي

أساسيات هندسة الأنسجة وتطبيقاتها فى علاج الجروح الجلدية والتنامها بواسطة الشيتوزان و الخلايا الجذعية

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[1]قسم الأنسجة والخلايا-كلية الطب البيطري-جامعة الزقازيق

تعد الجروح و القرح الجلدية من المشكلات الطبية واسعة الإنتشار بسبب الحوادث والحروق , خصوصا الجروح الكبيرة المزمنة و الحادة التي يصعب علاجها , ولا يتم التئامها بشكل طبيعي و تتسبب فى تشوه شكل الجلد , و فى كثير من الحالات يحدث تلوث للجرح و تعرضه للعدوى ومضاعفاتها الصحية الخطيرة, و لذلك فى الحالات الحرجة يتم اللجوء الي عمليات ترقيع الجلد من نفس المريض أو من متبرع وتقبل ما يترتب عليها من أعراض جانبية.

فمن خلال هذه الدراسة نلقي الضوء على تقنية هندسة الانسجة التي تعد أحد التطبيقات العلاجية الحديثة فى مجال الطب التجديدي من خلال استخدام الخلايا الجذعية لتسريع التئام الجرح و مقارنتها مع مركب الشيتوزان الذي يمتاز بخصائص طبية و علاجية متعددة و كذلك دراسة نتيجة الدمج بينهما لتخليق رقعة جلدية اصطناعية محاكاة للجلد الطبيعي فى الوظيفة و الحصول علي شكل تشريحي طبيعي لجلد مكتمل الوظائف الحيوية فى نهاية فترة العلاج و تسريع من عملية التئام الجرح لتجنب المخاطر المترتبة على الجروح المفتوحة و علاجها فى فترة زمنية قصيرة مقارنة بالطرق التقليدية, حيث تعد القولبة (السقالة) أحد تطبيقات هندسة الأنسجة لإعادة بناء أنسجة و أعضاء الجسم , وهذا القالب (السقالة) يمكن تصنيعه بطرق مختلفة باستخدام بوليميرات قابله للتحلل والإمتصاص من خلال الجسم و غير سامة , و يتم صبها و قولبتها لتحاكي الشكل و الخصائص التشريحية للعضو المراد تجديده.