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BIOSYNTHESIS OF PH RESPONSIVE SHAPE MEMORY HYDROGEL AND ITS BIOMEDICAL POTENTIAL

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Abstract

Multifunctional hydrogels combining the capabilities of cellular pH responsiveness and shape memory, are highly promising for the realization of smart membrane filters, controlled drug released devices, and functional tissue-engineering scaffolds. Free radical biocatalytic polymerization catalyzed by immobilized *Candida antarctica* lipase B was used to fabricate the pH-responsive and shape memory hydrogel using medium-chain-length poly-3-hydroxyalkanoates-co-polyethylene glycol methacrylate (PHA-PEGMA) as macromer. The accelerative wound healing potential of the biosynthesized smart PHA based hydrogel was evaluated herein. The thermal stability of the macromere highly depends on the PEGMA fraction from 10 to 50% (mass). Similarly, the change in PEGMA fraction was also found to highly influence the hydrogel's hydration rate (*r*) from 2.83 x 10^{-5} to 7.63 x 10^{-5} mL/s. The hydrogel's equilibrium weight swelling ratio (qe), protein release and its diffusion coefficient (Dm) were all found to be pH dependent. For example, increasing the phosphate buffer pH from 2.4 to 13 resulted in increased qe from 2 to 16 corresponding to the enlarging of network pore size (ξ) from 150 to 586 nm.The biomedical potential of the synthesized material based on its accelerative wound healing in rats was observed. Biochemical, histomorphometric and immunohistomorphometric analyses revealed a significant difference in area closure and re-epithelialization on days 7 and 14 in PPH or Intrasite® gel group compared to gum acacia or PEGMA-alone groups. Furthermore, wounds dressed with PPH or Intrasite® gel showed evident collagen deposition, enhanced fibrosis and extensively organized angiogenesis on day 14 compared to the negative control group. The findings suggested that topical application of PPH accelerated the rats' wound healing process by improving angiogenesis attributed to the increased microvessel density (MVD) and expressions of VEGF-A in tissue samples. Thus, PPH has been demonstrated to be effective in the treatment of cutaneous wounds in rats, and could be a potential novel agent in the management and acceleration of wound healing in humans and animals.

Key Words: Biocatalysis, Biomaterials, Biotechnology, Hydrogel, Wound-Healing.

Introduction

The effective control and treatment of some cutaneous wound under certain physiological or pathological conditions is problematic. This is due to the factors that interfere with the sequence of the wound healing process, leading to a delayed or impaired wound healing response, culminating in a revolting scar. As a result of these complications, impaired wound healing is an expensive and time-consuming undertaking. Inadvertently, it brings sociological, economical and public health burdens on the patients. Consequently, exploring affordable, effective and readily accessible alternative therapies for wound healing is currently an active area of research. Among such approaches are the use of phytochemical extracts (Al-Bayaty and Abdulla 2012, de Oliveira *et al.* 2013), carbohydrate based polymer composite (Nguyen *et al.* 2013).

Along this line, the biodegradability and biocompatibility of natural polymers such as poly-3-hydroxy alkanoates (PHAs) with the advantage of being produced by bacterial fermentation from renewable resources, or by *in vitro* green enzymatic catalysis strongly favor their applications in smart actuators, smart membrane filters, controlled drug released devices and tissue-engineering scaffolds etc.

Hydrogels made of amphiphilic polymer composites containing the biodegradable hydrophilic and lipophilic PHA are interesting due to their environment-sensitive micellar properties, such as temperature and pH responsiveness, excellent biocompatibility, lack of pro-inflammatory effects of polymeric systems and controlled capacity in protein release (Hennink *et al.* 1997). Since their first description by Guiseppi-Elie and Sheppard (1995), hydrogels are being exploited in several potential applications such as site-specific drug delivery devices (Schoener *et al.* 2012). In fact hydrogels made from biocompatible polyhydroxyalkanoates (PHA), polyamide and/or polyamino acids composites were reported to exhibit bio-mimetic properties especially in bioactuators, biosensors, smart ion exchange membranes, cell-adhesion or targeting, and their efficacy is well distinguished in cancer biology and innate immunity (Suresh 2007).

The use of PHAs based hydrogels in accelerative cutaneous wound healing presents several advantages over the commonly used alternatives. For example, the diverse structural composition of PHAs confers myriad of superior physico-mechanical properties. These include crystallinity, flexibility, thermal stability, piezoelectricity, porosity etc. Owing to these properties, the neat polymer or its composite could be suitably casted as film and used as superb adhesive bandage. The bioactivity of polyhydroxy- group has been reported Teramoto *et al.* (2008). Since the PHA backbone is adorned with numerous such groups, an inherent bioactivity could be perceived.

Despite their reported biomedical importance spanning from antimicrobial (Saad *et al.* 2012), target-guided devices (Yao *et al.* 2008), controlled drug release devices (Xiong *et al.* 2010), smart hydrogel (Gumel and Annuar 2014), to surgical implant (Türesin *et al.* 2001), research on the use of biocompatible and biodegradable PHAs in accelerative wound healing is very scarce (Bai *et al.* 2014).

In this study, mild-conditioned dual enzymatic catalysis was used to prepare a macromer and its respective smart hydrogel. The characterization of the said biomaterial were also studied and it accelerative wound healing activity was observed

2.2 Methods

2.2.1 Enzymatic synthesis of poly(3-hydroxyalkanoates)-*co*-(6-hydroxyhexanoate) macromer (PHA-PCL)

Bacterial mcl-PHAs was obtained according to previously reported methods(Gumel *et al.* 2012). The PHA sample (200 mg) was dissolved in a capped vial containing 10.0 ml toluene. To this solution, 3.0 ml of ε-caprolactone and 150 mg of immobilized enzyme (Novozym) were added. Then, the reaction mixture was incubated in an automatic orbital shaker (Labtech, Korea) at 50 (±1) °C, 250 rpm for 48 hours.

At the end of the reaction, the synthesized macromer was then extracted from the crude reaction mixture as reported somewhereelse (Gumel and Annuar 2014).

2.2.2 Synthesis of the poly(3-hydroxyalkanoates)-*co*-(6-hydroxyhexanoate) based hydrogel

The hydrogel film was synthesized by free radical polymerization as illustrated in Scheme 1. A solution of PHA-PCL macromer was prepared by dissolving the macromer (100 mg) in 1.5 ml chloroform and mixed with 1.0 ml solution of 20 % (w/w??) PEGMA in 50 mM physiological phosphate buffer (pH 7). PEGMA acted as a cross-linker. The mixture was sparged with nitrogen gas for 5 min. 120 µl of freshly prepared solutions of 10 % w/v APS and 100 µl of 20 % v/v TEMED that served as free radical initiators were sequentially added to the mixture. Following this, the reaction mixture was quickly poured into a 10 ml beaker (2.5 cm internal diameter) at 2 mm thickness and allowed to polymerize for 20 min at 25 °C . Thereafter, the synthesized hydrogel was freed out of impurities by sterilized water immersion followed by oven drying prior to experimental testing (Figure 1). Characterization of the synthesized biomaterial was observed as reported previously (Gumel *et al.* 2015).

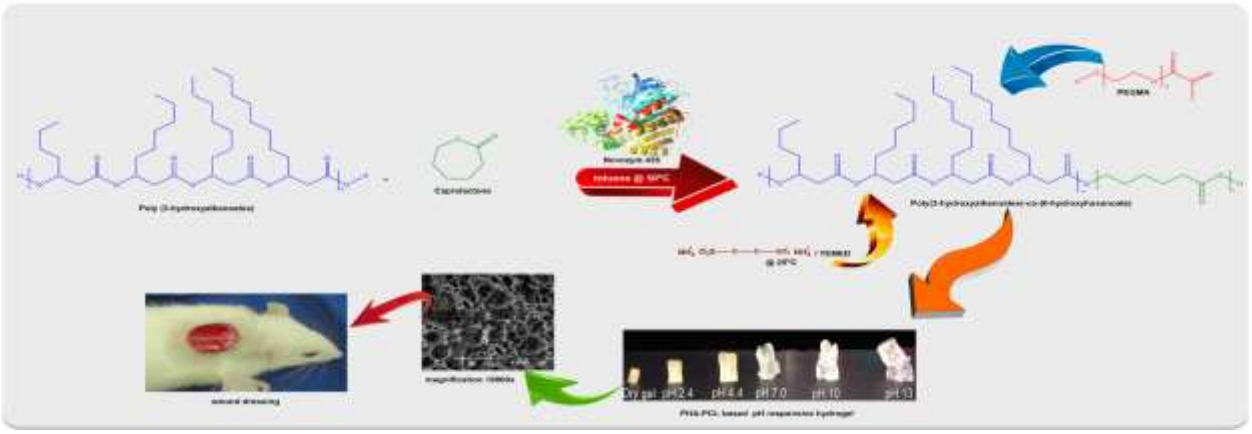

Figure 1 Schematic of PHA-PCL hydrogel biosynthesis and testing

2.2.6 Experimental Animal Model

The accelerative wound healing activity of PHA-PCL based hydrogel film was evaluated using rats model as reported in literature (Hajiaghaalipour *et al.* 2013). The experimental wound excision, wound dressing and wound area contracture were all observed according to literature (Kirker *et al.* 2002).

2.2.7 Wound Excision

The initial weight of each rat was recorded before the infliction of wound excision. After the weighing, the experimental wound excision was inflicted on the rat as described in literature (Al-Bayaty and Abdulla 2012).

2.2.10 Immunohistochemical analysis

The immunohistochemical staining for the determination of microvessel density (MVD) and expression of vascular endothelial growth factor A (VEGF-A) was performed according to the method of Zhang *et al.* (2010)

2.2.11 Wound tissue homogenate protein content

The total protein content of the wound tissue from each animal was determined according to the method described by Al-Bayaty and Abdulla (2012).

2.2.12 Estimation of connective tissue parameters

Tissue samples collected for the estimation of connective tissue parameters (hydroxyproline, hexosamine and hexuronic acid contents) were prepared according to the methods explained in literature (Murthy *et al.* 2013).

2.2.12.1 Hydroxyproline (HPR), Hexosamine (HXA) and Hexuronic acid (HUA) estimation

Wound tissue hydroxyproline content was estimated following the method described by Murthy *et al.* (2013) and (Gumel *et al.* 2015)

2.3 Statistical analysis

Statistical analysis was performed using Sigmaplot ver. 11.0 (SYSTAT^{TM}, USA). All experimental data were presented as average ± standard deviation and statistical significance were determined using ANOVA at 95% confidence interval.

3. Results

3.1 In vitro cytotoxicity assay

Human HeLa cell derivatives (WRL68) was used as a cellular model during the evaluation of the hydrogel's cytotoxicity following MTT reduction assay. With reference to control sample, the results demonstrated that increasing the hydrogel sample size up to 2.5 g did not caused a significant decrease in cell viability even after 72 hours incubation (96 % cell viability at 2.5 g hydrogel extract).

3.3 Percentage wound contraction

The percentage of wound contraction was measured at day 3, 7 and 14 . At the early phase of the wound healing (day 3), there was no significant difference between the negative control group and the treated groups. On day 7, the wound area in the whole samples was found to contract in comparison to the initial excision time. However, no significant difference was observed between the negative control group (10 %) and the secondary control (PH) group (13 %). In contrast to the negative control group, there was a significant difference ($p < 0.05$) in wound contracture of PPH (49 %) and Intrasite® (56 %) dressed groups. As the wound aged to day 14, the PH dressed group was observed to show marked increase in wound contraction (45 %) when compared to the gum acacia treatment group (30 %). At this time, the increase was found to be significantly different ($p < 0.05$) in both PPH (76 %) and Intrasite® dressed groups (80 %). It could be observed that PPH dressing showed comparable wound contraction performance to Intrasite® gel.

3.5 Immunohistochemical analysis

On day 14 post-excision, the MVD and the expression of VEGF-A were found to be at peak for all the sample tested (Figure). However, with reference to negative control (Figure 4 (a) and (e)) and PH dressed (Figure 4(b) and (f)) groups, the PPH dressed group revealed a significant ($p < 0.05$) increased in both MVD and VEGF-A expression (Figure 4 (c) and (g)). In fact, the immunohistomorphometric appearance of the PPH dressed micrographs indicated a normal wound healing process that was accompanied by high angiogenesis and VEGF-A expression to a level almost similar to that observed in the micrographs of the standard Intrasite® gel dressed group (Figure 4 (d) and (h)).

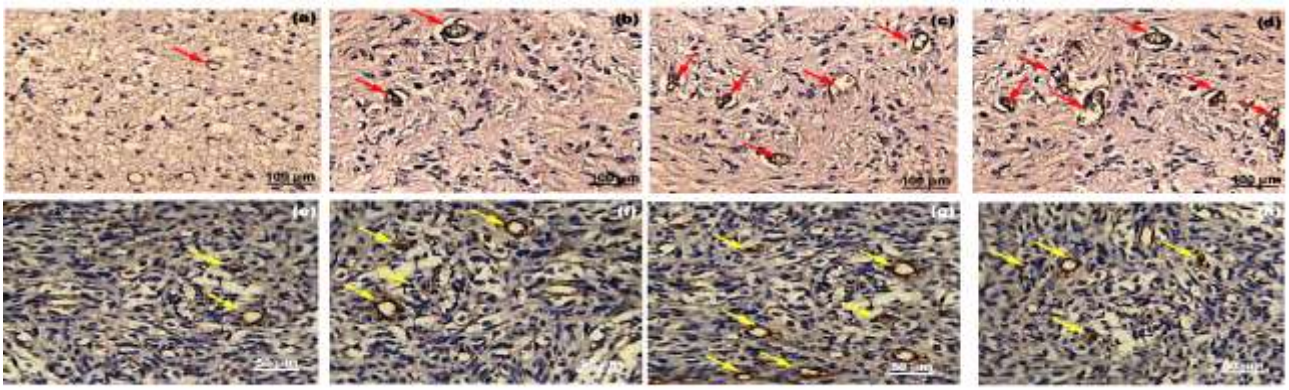

Figure 2. Immunohistochemical micrographs of excisional wound tissue on day 14 of dressing stained with anti-CD31 (*a-d*) and anti-VEGF-A (*e-h*) antibodies at 400 × magnification: (a) negative control (b) PH dressed group; (c) PPH dressed group; (d) Intrasite dressed group. VEGF-A stain (e) negative control group; (f) PH dressed group; (g) PPH dressed group and (h) Intrasite® gel dressed group. The *red arrow* depicts micro vessel density and *yellow arrow* denotes the VEGF-A expression.

3.6 Tissue homogenates protein content

The total protein content was found to significantly increase (p <0.05) in both the PPH (276 ±10.4 mg/ g tissue homogenates) and Intrasite® gel (308 ±12.3 mg/ g tissue homogenates) dressed groups as compared to the negative control group (Figure 6). In contrast, although there was a marked increase in protein content of PH dressed group (208 ±14.2 mg/ g tissue homogenates), it was not significant compared to the negative control group (189 ±19.3 mg/ g tissue homogenates). In general, higher protein level comparable to that of Intrasite® gel dressed group was observed in wounds dressed with PPH (Figure 6).

3.7 Connective tissue parameters

With reference to the data for negative control group, the HPR, HXA and HUA values were significantly (p < 0.05) increased in PH, PPH and Intrasite® dressed groups, respectively (Table 1). In comparison to the gum acacia control group, the HPR, HXA and HUA were significantly increased in PPH dressed group by 42 %, 59 % and 163 %, respectively. Murthy *et al.* (2013) reported a similar increase in HPR, HXA and HUA when investigating the wound healing activities of *Bacopa monniera* in rats.

Table 1 Connective tissue parameters on the effects of PHA-PCL hydrogel dressing on excisional wound healing

Dressing	Connective tissue parameters		
	Hydroxyproline *(µg/mg tissue hydrolysate)*	**Hexosamine** *(µg/mg tissue hydrolysate)*	**Hexuronic acid** *(µg/mg tissue hydrolysate)*
Negative control	148.9 ± 7.4	78.2 ± 2.9	19.5 ± 1.2
PH dressed	172.3 ± 8.6	98.3 ± 5.8	24.5 ± 2.4
PPH dressed	212.1 ± 12.2*	124.4 ± 6.2*	51.8 ± 2.6*
Intrasite® gel	228.6 ± 11.3*	127.5 ± 4.6*	53.4 ± 2.7*

Data values are expressed as average ± standard deviation of six (6) rats per each group. And asterisk (*) represents a significant (p < 0.05) difference when compared to negative control group.

4. Discussions

In this study, we evaluated the effects of topical dressing with chemo-enzymatically synthesized hydrogel based on biocompatible PHA-PCL composite on skin excision wounds in rats. In this research, *in vitro* MTT cytotoxicity evaluation revealed no significant difference (p > 0.05) in terms of WRL68 cellular proliferation and viability between the control group and the leachable residues of the composite polymer hydrogel samples even at longer exposure time (72 hours) and higher material weightage (2.5 g). This observation was found to be in agreement to other similar observations of high cellular viability (> 80%) following hydrogel cytotoxicity tests (Draye *et al.* 1998, Trudel and Massia 2002, Wu *et al.* 2008).

Histological examination of the excisional wound area revealed an observable increase in wound repair upon topical dressing with the synthesized hydrogels. Wound contraction was found to occur in the early days of post wound excision upon topical dressing. A dramatic increase in wound contraction was observed to occur within 7 to 10 days post excision. In fact, a significant increase (p < 0.05) in the percentage of wound contraction was observed in PPH and Intrasite® gel dressed groups on day 7 of post excision and the area was further reduced on day 14 post excision. Related decrease in the percentage of wound contracture upon dressing with aftamed and chlorine dioxide gels was reported in streptozotocin-induced diabetic rats (Al-Bayaty and Abdulla 2012). Similarly, significant increase in wound contracture was observed on day 7 in thermosensitive polyethyleneglycol triblock hydrogel (Lee *et al.* 2003). In another study, topical dressing with glycosaminoglycan hydrogel revealed no significant difference between the control group and the treated groups (Kirker *et al.* 2002). However, by day 7 post excision, the authors reported a significant increase in re-epithelialization (Kirker *et al.* 2002). Evidently, in this study, the increase in wound contracture corroborated with a marked increase in re-epithelialization in groups dressed with PPH and Intrasite® gels. In addition to re-epithelialization, the excision wounds revealed faster healing process due to the observed extensive increase in granulation tissue and reduced number of mononuclear inflammatory cells in these groups. Furthermore, a reduction in eosinophilic infiltration, increased neovascularization, restoration of adnexa, and pronounced fibrosis was also noted.

The micrographs obtained from the Mason's trichrome stain further supported these observations due to the presence of elevated levels of angiogenesis and collagen deposition. Angiogenesis increases the delivery of much need oxygen and nutrients for cellular proliferation and collagen deposition within the wound area. Impaired wound healing process is attributed low blood flow, which consequently resulted in lower oxygen supply and poor collagen deposition (Al-Bayaty and Abdulla 2012). However, influx of the macrophages and the increased neovascularization accompanied by strong collagen deposition in the granulation tissue could explain the mechanism underlying the topical dressing of PPH and Intrasite®

gel. Indeed, it has been hypothesized that wound treatment with bioactive agents that influence fibrosis, endothelial cytogenesis and proliferation, as well as increase the rate of granulation tissue development are amendable to accelerative rate of wound repair (Davidson *et al.* 1985, Grotendorst *et al.* 1985). Cellular contraction is hypothesized to be a more important factor than collagen deposition when it comes to the reduction of wound area in early phase of wound healing process (Majno *et al.* 1971). In this study, we found an apparent dependence of the percentage of wound area contraction on collagen deposition and angiogenesis. This observation is in agreement with the previous studies by Kant *et al.* (2015) and Shukla *et al.* (1999) who reported the increase in collagen deposition and angiogenesis to be among the main factors that enhanced wound healing activities. In fact, angiogenesis was claimed to be necessary for normal fibroblast and leukocyte functioning during wound healing process (Slavin 1996).

Occurrence of angiogenesis, presence of proliferating mesenchymal cells and influx of macrophages are among the major constituents of the granulation tissue (Davidson *et al.* 1985), inferred to provide substrates for the expression of re-epithelization inducers (Davidson *et al.* 1985, Al-Bayaty and Abdulla 2012). The observed poor re-epithelialization in the gum acacia and PH control groups could be attributed to the observed mediocre granulation tissue and less collagen deposition in wound tissue samples obtained from these groups.

Cytokines are low molecular weight proteins (≈ 4 to 60 kDa) and function mostly in cellular signaling (Diegelmann and Evans 2004). It has been hypothesized that cytokines achieved the function of cell signaling by altering the intracellular kinase activity and phosphorylation of major proteins consequently altering the cellular physiological status and cellular function (Debono 2000). Armed with cellular signaling ability, cytokines were reported to be the molecular time controlled switch between different phases of wound healing process (Debono 2000). During these processes, several cytokines activities are known to take place e.g. transforming growth factor beta (TGF-β) is known to be involved in wound healing and scar formation (O'Kane and Ferguson 1997). Similarly, platelet-derived growth factor (PDGF) that is attributed to pro-inflammatory cytokine protein of dimeric structure attracts leucocytes and fibroblasts to the healing wound (Slavin 1996). Furthermore, vascular endothelial growth factor (VEGF) which is also a dimeric protein and a heparin-binding endothelial growth factor is delineated to be specifically associated with vascular endothelial cells (Slavin 1996). Its binding sites on vascular endothelium were previously illuminated (Ferrara *et al.* 1991). In fact, *in situ* molecular hybridization have revealed the VEGF mRNA to be highly expressed and abundantly distributed in the areas of active vascular proliferation (Slavin 1996). Recent studies have reported a marked reduction in pig's wound angiogenesis, fluid accumulation, and granulation tissue formation upon application of neutralizing VEGF-A antibodies (Howdieshell *et al.* 2001, Werner and Grose 2003). Moreover, neutralization of VEGF was demonstrated to strongly inhibit angiogenic activity in human wound (Nissen *et al.* 1998). Finally, a pronounced reduction in angiogenesis and granulation tissue development was observed in murine cutaneous wound upon delivery of retroviral dominant-negative VEGFR-2 (Tsou *et al.* 2002).

In this study, we evaluated the microvessel density (MVD) and the expression of VEGF using immunohistochemical staining. As expected, with reference to the control and PH dressed groups, there was an observed marked increase in MVD and VEGF-A expression in both PPH and Intrasite® gel dressed samples. This clearly acceded to the prior literature reports and our own observation of neovascularization in H&E staining micrographs of the same samples. Similar report on enhanced wound healing process due to increased MVD and elevated expression of VEGF-A was reported (Kirker *et al.* 2002). During wound healing process, lipids and fatty acids are postulated to play an important role in the development of new micro-vessels and enhanced VEGF-A expression (Sellmayer *et al.* 1999, Kirker *et al.* 2002, Claria 2006). Previously, the bioactivity of polyhydroxy- compounds including PHA and PCL was established (Teramoto *et al.* 2008, Gumel *et al.* 2013). Since PPH is based on PHA-PCL copolymers composed of 3-hydroxy and 6-hydroxy fatty acids monomers, respectively, this could probably explain the underlying mechanism for the observed increase in MVD and elevated VEGF-A expression in PPH dressed group.

Conclusion

Enzymatically synthesized PHA-PCL macromers were cross-linked with PEGMA to give a bioactive hydrogel that can be aseptically sterilized and topically dressed on cutaneous wound in experimental rat models. Significant difference in area contracture and pronounced re-epithelialization was found on day 7 and 14 in the wounds dressed with the PPH and Intrasite® gel compared to those dressed with gum acacia or PEGMA alone. Additionally, those wounds dressed with PPH or Intrasite® gel showed marked collagen deposition, more fibrosis and extensively organized angiogenesis by day 14 compared to negative control or PH dressed groups. There were no significant differences between the negative control group and the PH dressed group on any day post wound-excision.

The findings suggested that topical application of the PPH helped to accelerate experimental rats wound healing by improving angiogenesis, which was attributed to the increased in MVD and expression of VEGF-A in tissue samples. Thus, PPH has been demonstrated to be effective in the treatment of cutaneous wounds in rats. It could be potentially employed as a novel agent for the management and acceleration of wound healing in human and animals.

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Conflict of Interest

The author declares that there is no existence of competing interests, financially or otherwise.

1. References

2. Al-Bayaty, F. and M. A. Abdulla (2012). "A comparison of wound healing rate following treatment with aftamed and chlorine dioxide gels in streptozotocin-induced diabetic rats." Evidence-Based Complementary and Alternative Medicine **2012**.

3. Bai, M. Y., T. C. Chou, J. C. Tsai and W. C. Yu (2014). "The effect of active ingredient-containing chitosan/polycaprolactone nonwoven mat on wound healing: In vitro and in vivo studies." Journal of Biomedical Materials Research Part A **102**(7): 2324-2333.

4. Claria, J. (2006). "Regulation of cell proliferation and apoptosis by bioactive lipid mediators." Recent patents on anti-cancer drug discovery **1**(3): 369-382.

5. Davidson, J. M., M. Klagsbrun, K. E. Hill, A. Buckley, R. Sullivan, P. S. Brewer and S. C. Woodward (1985). "Accelerated wound repair, cell proliferation, and collagen accumulation are produced by a cartilage-derived growth factor." The Journal of cell biology **100**(4): 1219-1227.

6. de Oliveira, A. P., E. d. S. Franco, R. Rodrigues Barreto, D. P. Cordeiro, R. G. de Melo, C. M. F. de Aquino, P. L. de Medeiros, T. G. da Silva, A. J. d. S. Góes and M. B. d. S. Maia (2013). "Effect of semisolid formulation of persea americana mill (avocado) oil on wound healing in rats." Evidence-Based Complementary and Alternative Medicine **2013**.

7. Debono, R. (2000). Histological and immunohistochemical studies of excisional wounds in the rat with special reference to the involvement of the hair follicles in the wound healing process, Durham University.

8. Diegelmann, R. F. and M. C. Evans (2004). "Wound healing: an overview of acute, fibrotic and delayed healing." Front Biosci **9**(1): 283-289.

9. Draye, J.-P., B. Delaey, A. Van de Voorde, A. Van Den Bulcke, B. De Reu and E. Schacht (1998). "In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films." Biomaterials **19**(18): 1677-1687.

10. Ferrara, N., K. A. Houck, L. B. Jakeman, J. Winer and D. W. Leung (1991). "The vascular endothelial growth factor family of polypeptides." Journal of cellular biochemistry **47**(3): 211-218.

11. Grotendorst, G. R., G. Martin, D. Pencev, J. Sodek and A. Harvey (1985). "Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats." Journal of Clinical Investigation **76**(6): 2323.

12. Guiseppi-Elie, A. and J. N. Sheppard (1995). Conferring biospecificity to electroconductive polymer-based biosensor devices. ACS Northeast Regional Meeting (NERM), Rochester, NY, American Chemical Society.

13. Gumel, A. M. and M. S. M. Annuar (2014). "Poly-3-hydroxyalkanoates-co-polyethylene glycol methacrylate copolymers for pH responsive and shape memory hydrogel." Journal of Applied Polymer Science **131**(23).

14. Gumel, A. M., M. S. M. Annuar and Y. Chisti (2013). "Recent Advances in the Production, Recovery and Applications of Polyhydroxyalkanoates." Journal of Polymers and the Environment **21**(2): 580-605.

15. Gumel, A. M., M. S. M. Annuar and T. Heidelberg (2012). "Biosynthesis and Characterization of Polyhydroxyalkanoates Copolymers Produced by *Pseudomonas putida* Bet001 Isolated from Palm Oil Mill Effluent." PLoS ONE **7**(9): e45214.

16. Gumel, A. M., M. R. M. Razaif-Mazinah, S. N. S. Anis and M. S. M. Annuar (2015). "Poly (3-hydroxyalkanoates)-co-(6-hydroxyhexanoate) hydrogel promotes angiogenesis and collagen deposition during cutaneous wound healing in rats." Biomedical Materials **10**(4): 045001.

17. Hajiaghaalipour, F., M. Kanthimathi, M. A. Abdulla and J. Sanusi (2013). "The Effect of Camellia sinensis on Wound Healing Potential in an Animal Model." Evidence-Based Complementary and Alternative Medicine **2013**.

18. Hennink, W. E., O. Franssen, W. N. E. van Dijk-Wolthuis and H. Talsma (1997). "Dextran hydrogels for the controlled release of proteins." Journal of Controlled Release **48**(2–3): 107-114.

19. Howdieshell, T. R., D. Callaway, W. L. Webb, M. D. Gaines, C. D. Procter, J. S. Pollock, T. L. Brock and P. L. McNeil (2001). "Antibody neutralization of vascular endothelial growth factor inhibits wound granulation tissue formation." Journal of Surgical Research **96**(2): 173-182.

20. Kant, V., A. Gopal, D. Kumar, N. N. Pathak, M. Ram, B. L. Jangir, S. K. Tandan and D. Kumar (2015). "Curcumin-induced angiogenesis hastens wound healing in diabetic rats." Journal of Surgical Research **193**(2): 978-988.

21. Kirker, K. R., Y. Luo, J. H. Nielson, J. Shelby and G. D. Prestwich (2002). "Glycosaminoglycan hydrogel films as bio-interactive dressings for wound healing." Biomaterials **23**(17): 3661-3671.

22. Lee, P.-Y., Z. Li and L. Huang (2003). "Thermosensitive hydrogel as a Tgf-β1 gene delivery vehicle enhances diabetic wound healing." Pharmaceutical research **20**(12): 1995-2000.

23. Majno, G., G. Gabbiani, B. Hirschel, G. Ryan and P. Statkov (1971). "Contraction of granulation tissue in vitro: similarity to smooth muscle." Science **173**(3996): 548-550.

24. Murthy, S., M. Gautam, S. Goel, V. Purohit, H. Sharma and R. Goel (2013). "Evaluation of In Vivo Wound Healing Activity of Bacopa monniera on Different Wound Model in Rats." BioMed research international **2013**.

25. Nguyen, V. C., V. B. Nguyen and M.-F. Hsieh (2013). "Curcumin-loaded chitosan/gelatin composite sponge for wound healing application." International Journal of Polymer Science **2013**.

26. Nissen, N. N., P. Polverini, A. E. Koch, M. V. Volin, R. L. Gamelli and L. A. DiPietro (1998). "Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing." The American journal of pathology **152**(6): 1445.

27. O'Kane, S. and M. W. Ferguson (1997). "Transforming growth factor βs and wound healing." The international journal of biochemistry & cell biology **29**(1): 63-78.

28. Saad, G. R., M. A. Elsawy and M. Z. Elsabee (2012). "Preparation, Characterization and Antimicrobial Activity of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate)-g-Poly (N-vinylpyrrolidone) Copolymers." Polymer-Plastics Technology and Engineering **51**(11): 1113-1121.

29. Schoener, C. A., H. N. Hutson and N. A. Peppas (2012). "pH-responsive hydrogels with dispersed hydrophobic nanoparticles for the oral delivery of chemotherapeutics." Journal of Biomedical Materials Research Part A.

30. Sellmayer, A., N. Hrboticky and P. C. Weber (1999). "Lipids in vascular function." Lipids **34**(1): S13-S18.

31. Shukla, A., A. M. Rasik and B. N. Dhawan (1999). "Asiaticoside-induced elevation of antioxidant levels in healing wounds." Phytotherapy Research **13**(1): 50-54.

32. Slavin, J. (1996). "The role of cytokines in wound healing." The Journal of pathology **178**(1): 5-10.

33. Suresh, S. (2007). "Biomechanics and biophysics of cancer cells." Acta Materialia **55**(12): 3989-4014.

34. Teramoto, N., N. D. Sachinvala and M. Shibata (2008). "Trehalose and trehalose-based polymers for environmentally benign, biocompatible and bioactive materials." Molecules **13**(8): 1773-1816.

35. Trudel, J. and S. Massia (2002). "Assessment of the cytotoxicity of photocrosslinked dextran and hyaluronan-based hydrogels to vascular smooth muscle cells." Biomaterials **23**(16): 3299-3307.

36. Tsou, R., C. Fathke, L. Wilson, K. Wallace, N. Gibran and F. Isik (2002). "Retroviral delivery of dominant-negative vascular endothelial growth factor receptor type 2 to murine wounds inhibits wound angiogenesis." Wound repair and regeneration **10**(4): 222-229.

37. Türesin, F., I. Gürsel and V. Hasirci (2001). "Biodegradable polyhydroxyalkanoate implants for osteomyelitis therapy: in vitro antibiotic release." Journal of Biomaterials Science, Polymer Edition **12**(2): 195-207.

38. Werner, S. and R. Grose (2003). "Regulation of wound healing by growth factors and cytokines." Physiological reviews **83**(3): 835-870.

39. Wu, D.-Q., T. Wang, B. Lu, X.-D. Xu, S.-X. Cheng, X.-J. Jiang, X.-Z. Zhang and R.-X. Zhuo (2008). "Fabrication of supramolecular hydrogels for drug delivery and stem cell encapsulation." Langmuir **24**(18): 10306-10312.

40. Xiong, Y.-C., Y.-C. Yao, X.-Y. Zhan and G.-Q. Chen (2010). "Application of polyhydroxyalkanoates nanoparticles as intracellular sustained drug-release vectors." Journal of Biomaterials Science, Polymer Edition **21**(1): 127-140.

41. Yao, Y.-C., X.-Y. Zhan, J. Zhang, X.-H. Zou, Z.-H. Wang, Y.-C. Xiong, J. Chen and G.-Q. Chen (2008). "A specific drug targeting system based on polyhydroxyalkanoate granule binding protein PhaP fused with targeted cell ligands." Biomaterials **29**(36): 4823-4830.

42. Zhang, Z., S. Wang, Y. Diao, J. Zhang and D. Lv (2010). "Fatty acid extracts from Lucilia sericata larvae promote murine cutaneous wound healing by angiogenic activity." Lipids Health Dis **9**: 24.