A PROSPECTIVE MULTICENTER SINGLE-BLINDED RANDOMIZED CONTROLLED TRIAL TO EVALUATE THE EFFICACY OF CHITOSAN SPONGE VERSUS ALLEVYN ON EXUDATIVE WOUNDS

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III. PREFACE

Wounds are a major problem in Malaysia and worldwide. The bulk of this are chronic wounds from diabetic ulcers, vascular ulcers and pressure sores. These demand a lot of our healthcare resources especially when the cost of wound care devices including dressings are rising. According to the current Director General of health of Malaysia, Dato Dr Noor Hisham Abdullah, in a press conference in Kuching, Sarawak recently, a staggering RM1 million is spent every month by seven hospitals nationwide in 2013 to manage patients' wounds due to costly and advanced dressing materials. Most of this advanced dressings are imported from overseas. One of the best solution to this problem is to develop our own dressings which are affordable but give the same or even superior results.

Chitosan-based dressing has a great potential that may fit the above descriptions. It can be manufactured locally and designed in such a way to meet the physical as well as spiritual requirements of the local population, for example by avoiding porcine or bovine contents. The savings could then be channelled towards other areas of healthcare such as cancer research.

Obviously, in the early stages, there will be considerable costs involved to carry out high quality research and development but in the long run, it will be a worthwhile investment for the sake of our future healthcare.

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V. ABBREVIATIONS

DD	Degree of deacetylation
Mw	Molecular weight
GAG	Glycosaminoglycan
PDGF	Platelet-derived growth factor
TGF-β / -α	Transforming growth factor beta / alpha
EDGF	Endothelial-derived growth factor
VEGF	Vascular endothelial growth factor
FGF	Fibroblast growth factor
TNF	Tumour necrosis factor
ILF	Insulin-like growth factor
KGF	Keratinocyte growth factor
IL-1	Interleukin one
NO	Nitric oxide
USA	United States of America
PMN	Polymorphonuclear leukocytes
RCT	Randomized controlled trial
HUSM	Hospital Universiti Sains Malaysia
PPUKM	Pusat Perubatan Universiti Kebangsaan Malaysia
HKL	Hospital Kuala Lumpur
AMREC	Advanced Materials Research Centre
SIRIM	Standard and Industrial Research Institute of Malaysia
EO	Ethylene oxide
ISO	International Standards Organization
NIH	National Institute of Health
ANCOVA	Analysis of Covariance
ICC	Intraclass Correlation Coefficients
SD	Standard deviation
CI	Confidence interval

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IX. ABSTRAK

Sejak pertama kali ditemui oleh Henri Braconnot pada tahun 1811, kitosan telah menarik banyak minat di kalangan penyelidik kerana banyak sifat-sifat biologi bergunanya seperti pembekuan darah, anti-mikrobial dan kebolehan menyembuh luka, serta ia adalah bioserasi dan terbiodegradasikan. Unsur induknya, chitin, banyak terdapat dalam alam semula jadi dan boleh didapati dalam kulat, kulit luar haiwan krustasia dan banyak haiwan dan tumbuh-tumbuhan lain. Salah satu aplikasi perubatan berpotensi kitosan adalah sebagai bahan pembalut luka. Span kitosan dihasilkan melalui proses mengeringsejukbeku serbuk kitosan gred farmaseutikal yang dilarutkan di dalam 1% asid asetik, 20% gliserol dan campuran natrium bikarbonat.

Objektif utama kajian klinikal ini adalah untuk membandingkan keberkesanan span kitosan dengan Allevyn, yang sudah dikomersialkan sebagai pembalut luka berair, dari segi penyembuhan atau pertumbuhan kulit. Objektif lain termasuk perbandingan penyediaan permukaan luka, pengalaman pesakit, praktikaliti penggunaan pembalut luka dan penentuan apa-apa tindak balas yang buruk oleh pembalut luka. Hipotesis kajian adalah bahawa terdapat perbezaan yang signifikan dalam keberkesanan dan sifat-sifat penyembuhan antara span kitosan dan Allevyn sebagai pembalut untuk luka berair.

Sebanyak 72 pesakit dari 3 buah hospital berbeza dirawakkan ke dalam kajian ini antara Julai 2012 dan Ogos 2014 (24 bulan). Enam puluh (83.4%) melengkapi kajian dan telah sama-sama dibahagikan kepada kumpulan kitosan dan Allevyn.

Dua belas (16.6%) telah dihentikan, terutamanya kerana tidak mematuhi protokol kajian. Pesakit-pesakit dinilai secara klinikal dan simptom mereka direkodkan setiap 3 hari sepanjang tempoh rawatan. Data seperti saiz tisu granulasi dan tisu nekrotik, tahap rembesan, lekatan, kemudahan dan kesakitan semasa pembuangan pembalut, bau dan kegatalan diukur dan direkodkan. Peratusan pertumbuhan kulit diukur oleh 2 penyiasat yang dibutakan kepada kajian melalui siri gambar yang diambil pada permulaan, pertengahan dan akhir rawatan.

Hasil kajian menunjukkan bahawa span kitosan mempunyai peratusan lebih tinggi purata pertumbuhan kulit daripada Allevyn, tetapi perbezaannya tidak signifikan secara statistik ($p = 0.072$). Kadar pembentukan tisu granulasi dan pengurangan tisu nekrotik juga setanding bagi kedua-dua kumpulan. Tiada perbezaan yang signifikan didapati antara kumpulan dari segi pengalaman pesakit dan prestasi pembalut luka, kecuali bau luka pada Hari ke-24, di mana kitosan adalah jauh lebih buruk ($p = 0.02$). Tiada tindak balas serius atau alergi yang dikaitkan dengan span kitosan.

Kesimpulannya, tidak ada perbezaan yang signifikan dalam keberkesanan dan ciri-ciri penyembuhan di antara span kitosan dan Allevyn sebagai pembalut untuk luka berair. Oleh itu span kitosan mempunyai potensi sebagai pembalut luka berair yang setanding dengan pembalut luka komersial seperti Allevyn.

VIII. ABSTRACT

Since its first discovery by Henri Braconnot in 1811, chitosan has attracted a lot of interest among researchers due to its many useful biological properties such as haemostatic, anti-microbial and wound healing abilities, as well as being biocompatible and biodegradable. Its parent element, chitin, is abundant in nature and can be found in fungi, exoskeleton of crustaceans and many other animals and plants. One of the potential medical applications of chitosan is as a wound dressing material. Chitosan sponge is produced by lyophilisation of pharmaceutical grade chitosan powder dissolved in 1% acetic acid, 20% glycerol and sodium bicarbonate mixture.

The main objective of this clinical study was to compare the efficacy of chitosan sponge against an already commercialised Allevyn foam dressing on exudative wounds in terms of healing or epithelization. Other objectives include comparison of wound bed preparation, patients' experience, practicality of the dressing and determination of any adverse reaction to the dressing. The research hypothesis was that there are significant differences in efficacy and healing properties between chitosan sponge and Allevyn as dressings for exudative wounds.

A total of 72 subjects from 3 different hospitals were randomized into the study between July 2012 and August 2014 (24 months). Sixty (83.4%) completed the study and were equally divided into the chitosan and Allevyn groups. Twelve (16.6%) were discontinued, mainly due to non-compliant with the study

protocol. The subjects were assessed clinically and their symptoms recorded every 3 days throughout the treatment duration. Data such as size of granulation and necrotic tissues, level of exudates, adherence, ease and pain on removal, odour, itchiness, were measured and recorded. Percentages of epithelization were measured by 2 blinded investigators via a series of photos taken at the start, middle and end of the treatments.

The results showed that chitosan sponge had a higher mean percentage of epithelization than Allevyn but the difference was not statistically significant ($p = 0.072$). The rate of granulation tissue formation and reduction of necrotic tissue were also comparable between the 2 groups. No significant difference was found between the groups in terms of patients' experience and performance of the dressings, apart from wound odour on Day 24, where chitosan was significantly worse ($p = 0.02$). There was no allergic or serious adverse reaction associated with chitosan sponge.

In conclusion, there is no significant difference in efficacy and healing properties between chitosan sponge and Allevyn as dressings for exudative wounds. Hence, chitosan sponge has the potential to become as good an exudative wound dressing as the commercialised Allevyn.

A Prospective Multicenter Single-Blinded Randomized Controlled Trial to Evaluate the Efficacy of Chitosan Sponge versus Allevyn® on Exudative Wounds.

1.0 Introduction and literature review

1.1 Research background

Chitosan was first discovered by a French chemist and pharmacist, Henri Braconnot, in 1811. He observed that a certain substance (chitin) found in mushrooms did not dissolve in sulfuric acid (Labrude and Becq, 2003). Later on, chitin was found in the exoskeleton of crustaceans (such as crabs and shrimps). Chitin is the second most abundant naturally occurring polysaccharide after cellulose (Rinaudo, 2006). Chitosan is a cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine residues. It is a biodegradable, nontoxic, complex carbohydrate derivative of chitin (poly-N-acetyl-D-glucosamine) obtained by the alkaline, partial deacetylation of chitin. In general, the term chitosan is applied when the extent of deacetylation is above 70% and the generic term chitin is used when the degree of deacetylation is below 20%. These various grades of chitosan, differing in the degree of deacetylation (DD), have varying effects on its clinical applications (Yang et al., 2008).

Chitosan has many useful biological properties such as haemostatic, acesodyne (analgesic) and bacteriostatic. These properties make chitosan a potentially good material for wound dressing. In addition, studies have shown that it aids wound

healing and reduces scarring. It is also biocompatible and biodegradable (Chun-Mei Deng, 2007, Sashiwa et al., 1990).

1.2 Production of chitosan

Generally, chitosan produced from mushrooms present a narrow molecular weight distribution compared to chitosan produced from shrimps. A non-animal source is considered to be safer for biomedical and healthcare uses.

While chitin occurs in fungi, diatoms, nematodes, arthropods, and a great number of other animals and plants, commercial exploitation has focused on a limited number of species for chitin extraction (Hayes et al., 2008). Shrimps, crabs, lobsters, krill, and squid wastes from the marine processing industry have become the major resource used today (Teng et al., 2001).

1.3 Chemical structure of chitosan

Chitosan is usually prepared by the deacetylation of chitin. The conditions used for deacetylation will determine the average molecular weight (Mw) and degree of deacetylation (DD). The structure of chitosan is very similar to that of cellulose [made up of b (1-4)-linked D-glucose units], in which there are hydroxyl groups at C-2 positions of glucose rings. Chitosan is a linear copolymer polysaccharide consisting of b (1-4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units (Figure 1b).

The properties, biodegradability, and biological role of chitosan are frequently dependent on the relative proportions of N-acetyl-D-glucosamine and D-glucosamine residues. The term chitosan is used to describe a series of polymers of different Mw and DD, defined in terms of the percentage of primary amino groups in the polymer backbone. The DD of typical commercial chitosan is usually between 70 and 95%, and the Mw between 10 and 1,000 kDa.

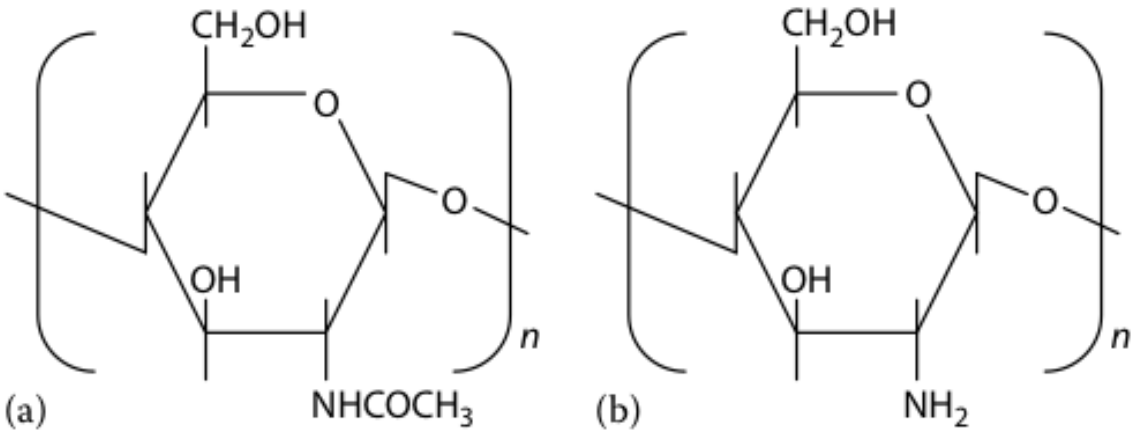

Figure 1. Molecular structures of (a) chitin and (b) chitosan.

1.4. Chitosan as biomaterial

Chitosan and its derivatives in the form of micro- and nanoparticles, composites, membranes and scaffolds have many applications as a biomaterial. Recent studies reported its use in drug and gene delivery, elaborated diagnostics, and surgical aids such as anti-adhesion gels. It has also been reported as a promising biomaterial for tissue engineering scaffold. This is due to its structure where the polysaccharide

unit of chitosan resembles the structure of glycosaminoglycans (GAG), which are a major component of extracellular matrix of bone and cartilage. In addition, the cationic nature of chitosan facilitates pH-dependent interaction with anionic GAGs, proteoglycans and other negatively charged molecules. This makes chitosan suitable in various shapes and sizes such as porous scaffolds, and hydrogels for interactions with growth factors, receptors and adhesion proteins. The physical and mechanical properties of chitosan can be improved by using graft copolymerization and crosslinking. For example, upon hydrogenation with simple aldehydes, chitosan produces N-alkyl chitosan. The physicochemical and biological properties as well as conformational structures of chitosan are therefore very effective for biomedical applications (Dutta et al., 2011).

1.5 Wound healing

Knowledge and understanding of the cellular and biomechanical components of wound healing has expanded significantly over the past four decades. Previously the process of healing was thought to be a passive process. This theory was summed up by Ambroise Pare, when he said, “I dressed the wound, God heals it” (Pare 1951).

It is now generally accepted that this is not exactly the case. Wound healing can be accelerated and enhanced by the use of specific wound care or dressing techniques and products (Cooper, 1990).

Wound healing can be divided into three phases: 1) Inflammatory, 2) Proliferative and 3) Remodelling. The phases are not distinct but rather a continuum with variable degrees of overlap (Hunt, 1990, Diegelmann and Evans, 2004).

1.5.1 Inflammation phase

The inflammatory phase starts immediately after wounding. This generally lasts up to 4 to 6 days of injury. The initial aim is haemostasis which is achieved through smooth muscle contraction of damaged blood vessels, activation and aggregation of platelets at the injured vessel wall. The platelets release clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β). (LaVan and Hunt, 1990, Falcone and Caldwell, 1990)

The other aims of the inflammatory phase are the removal of foreign body, microorganism and other contaminants. Neutrophils (also known as polymorphonuclear leukocytes) migrate from the surrounding microvasculature into the wound and start to phagocytose debris, foreign body, devitalized tissues and microorganism. Although neutrophils appear first, they are short-lived, lasting about six hours. Later, circulating monocytes infiltrate the wound extracellular matrix and replace neutrophils function for clearing the wound of the microbials, foreign materials and necrotic tissues. The circulating monocytes, known as macrophage, migrate into the wound and play a key role for the wound healing to progress in an organized manner by secreting and modulating multiple enzymes

and cytokines. Among them are collagenases, which debride the wound; platelet-derived growth factor (PDGF), transforming growth factor (TGF-α, TGF-β), endothelial-derived growth factor (EDGF), fibroblast growth factor (FGF), tumour necrosis factor (TNF), insulin-like growth factor (ILF) and many more which stimulate fibroblasts (produce collagen), promote angiogenesis and stimulates keratinocytes.

1.5.2 Proliferative phase

The proliferative phase can begin as early as 24 to 48 hours of injury and generally lasts up to 2 weeks. During this building portion of healing, angiogenesis, collagen deposition, granulation tissue formation and epithelization occur. Angiogenesis is marked by endothelial cell migration and capillary formation. The critical part for wound healing is the migration of the capillaries into the wound bed. These will supply the nutrients required in the granulation and tissue deposition phase, without which will result in an unhealed or chronic wound. Fibroblasts migrate into the wound site and once activated, synthesize a provisional matrix composed of collagen type III, GAGs, and fibronection. Wound fibroblasts also transform into myofibroblasts for wound contraction. In addition, for the healing to progress, the inflammatory reaction must also be controlled. The inflammatory cells, angiogenesis and granulation tissue decrease as the collagen deposition increases progressively.

1.5.2.1 Granulation and epithelization

The presence of healthy granulation tissue and its increase in the wound bed points towards healing. It is also considered a good marker of progress. However, further healing can be impeded due to various factors and the wound may remain static for prolonged periods. Nevertheless, the wound bed may be suitable for grafting or for the use of novel treatment modalities such as tissue-engineered skin substitutes or growth factors.

Epithelization may begin almost immediately after an injury, initiated by the keratinocytes at the wound edges or keratinocytes from skin appendages that are present in the wound (Ferreira et al.2009). These keratinocytes lose their connections to the adjacent cells and migrate over the wound surface. Their movement and migration across the wound is aided by actin filaments (Hamill et al., 2013). These single layer of cells spread in all directions until they come into contact with other migrating keratinocytes. This process is called contact inhibition (Wang et al., 2012). When contact inhibition is achieved, connections between the keratinocytes are reestablished (Odland and Ross, 1968). The keratinocytes continue to proliferate and form a multi-layered neo-epidermis that eventually gets covered with keratin.

1.5.3 Remodelling phase

The hallmark of the remodeling phase is the deposition of collagen in an organized network. Wound strength will be compromised if there are problems with matrix deposition. Conversely, hypertrophic scar or keloid will result if there is excess

collagen synthesis. The remodeling phase begins about one week of injury and may last up to a year. Net synthesis of collagen during this phase is not only from increased fibroblasts but also from increased in its production per cell and this will continue for at least 4 to 5 weeks (Diegelmann, 2003, Madden and Smith, 1970).

The initial collagen laid down is thin and orientated parallel to the skin. More mature type I collagen is deposited and organized along the stress lines in place of the initial collagen which is reabsorbed over time. This results in an increase tensile strength of the wound. However, granulation tissue collagen has thinner fiber size and is biochemically different from that of uninjured skin due to the hydroxylation and glycosylation of lysine residues in the former (Forrest, 1983).

Scar collagen will never become as organized as that of normal skin and the wound strength increases quickly within 1 to 6 weeks but will never return to the original state. The strength usually plateaus up to 1 year following the initial trauma. The number of fibroblasts decreases and the dense capillary network regresses. The tensile strength in the scar is about 30% when compared with normal skin. After about 3 weeks, an increase in breaking strength occurs due to crosslinking. Crosslinking is also responsible for wound contraction, brittle and less elastic scar. Histologically, the epidermo-dermal junction lacks the rete pegs in healed wounds which results in a fragile neoepidermis that easily avulses even with minor trauma (Sabiston and Townsend, 2012).

1.6 History of wound dressing

Wound dressing is "a material applied to protect a wound" that favours its healing. The evolution of wound dressings can be traced as far back as 1700 BC. In the Edwin Smith Papyrus, one of the oldest medical manuscripts, there are frequent references to fabrics used for bandages and dressings and the importance of certain aspects of treatment such as the drainage of deep or contaminated wounds. Hippocrates (circa 400 BC) advocated keeping wounds dry to promote healing. In contrast, Galen (circa 200 AD), a prominent Greek physician, emphasized his theory of "laudable pus" for the treatment of wounds. He postulated that wounds needed to suppurate in order to heal secondarily. His writings were adopted as basis of medical practice by the Romans and elsewhere for centuries. Theodoric (1266 AD) was the first who dared to challenge Galen's theory and emphasized the avoidance of "laudable pus" (Meade, 1968).

The concept of moist environment is better for wound healing were proposed by Winter in 1962. His experiment on pigs showed faster epithelization in wounds that were kept moist than those that were left exposed to dry and form scabs (Winter, 1962). A similar study in humans was also done in favour of moist over dry environment for wound healing (Hinman and Maibach, 1963).

1.7 Wound exudate

Both the studies mentioned above used occlusive type dressings to maintain the moist environment. The moisture is generally provided by fluid that has exuded out of a tissue or its capillaries from the wound bed due to injury or inflammation once haemostasis has been achieved. Exudate consists mainly of water but it also has electrolytes, nutrients, proteins, inflammatory mediators, proteases, growth factors, white blood cells and platelets. Healthy exudate is usually pale amber in colour, odourless and has a watery consistency (Thomas, 2010). This exudate is generally produced throughout the wound healing process and if not managed properly can have negative effects on wound healing. The wound may macerate if excessive exudate is retained in the wound but on the other hand, if the wound is too dry, healing will also be impeded. Foam or sponge dressings have become the treatment of choice for exudative wounds as they promote moisture balance at the wound interface through controlled absorption and evaporation.

Currently, there is no “one size fits all” dressing that can be used for all types wounds. Hence, there is a wide variety of dressings available, each with its own advantages and disadvantages. Wound dressings are generally classified as passive, interactive or bioactive (advance). The latter contains active compounds which aid wound healing of which chitosan biomaterial falls into.

1.8 Chitosan in medical applications

Chitosan-based medical products have been in the market for some time especially in Asian countries. These are typically for local market having passed through the strict regulatory requirement of individual countries where the products are sold. Concurrently, there have been many published in vitro and animal studies exploiting the desirable properties of chitosan in medical applications (Dai et al., 2011). However, similar or parallel clinical studies on chitosan have been limited. The three main properties of chitosan that have been of great interest are its haemostatic, antimicrobial and wound healing properties. In addition, chitosan has been used as carrier for drug-delivery in devices such as wound dressing that could enhance the effects of its properties stated earlier.

1.8.1 Haemostatic effects of chitosan

The backbone of chitosan is positively charged and therefore can bind with the negatively charged red blood cell membranes allowing for rapid clotting of blood (Rao and Sharma, 1997). A few chitosan-based products in the form of gauze, granules and bandages have gained regulatory approval in the USA for use as haemostatic devices such as Celox and HemCon. Several studies and clinical case reports have been published on their usage in civilian and military subjects (Millner et al., 2009, Kozen et al., 2008, Pozza and Millner, 2011).

1.8.2 Antimicrobial effects of chitosan

Chitosan has been extensively investigated as an antimicrobial agent for preventing and treating infections. The molecular weight, degrees of deacetylation (DD), the physical state of chitosan, and the ionic strength and pH of the dissolving medium can influence the antimicrobial properties of chitosan.

The exact mechanisms of the antimicrobial actions of chitosan are still uncertain. It has been proposed that disruption of microbial membrane or alteration in the cell permeability occur as a result of interaction between between positively charged chitosan molecules and negatively charged microbial cell membranes (Rabea et al., 2003, Raafat et al., 2008). Bacterial agglutination or suspension may also result when the polycationic chitosan binds to the negatively charged bacterial surface depending on its concentration (Rabea et al., 2003).

1.8.3 Wound healing effects of chitosan

As in the antimicrobial effects, the wound healing effects of chitosan could also be influenced by its molecular weight, deacetylation degree (DD) and its physical state (Alsarra, 2009, Azad et al., 2004). Chitosan has been shown to positively influence all the different stages of wound healing. It enhances the functions of inflammatory cells such as macrophages, polymorphonuclear leukocytes (PMN), fibroblasts and osteoblasts (Ueno et al., 2001, Peluso et al., 1994, Howling et al., 2001, Klokkevold et al., 1996). It has also been reported in an animal study that wound contraction was increased and breaking strength in the incision wound was significantly increased (Qin et al., 2010).

1.8.4 Analgesic effects of chitosan

The analgesic effect of chitosan could be explained by the ability of its amino group to absorb proton ions reducing the acidity in inflamed areas. Chitin on the other hand acts by absorption of bradykinin which does this more extensively than chitosan particles (Okamoto et al., 2002).

1.9 History of foam dressing

The first foam-like materials used in medicine were naturally occurring marine sponges. These were widely used in Europe and Middle East during the middle ages. The sponges were impregnated with chemicals and inserted in nostrils of patients to induce sleep during anaesthesia. Until the end of the 19th Century, sponges were also used as absorbents, cleaning aids and haemostats during surgery but the difficulty in sterilizing them and the tendency to adhere to wound surfaces resulted in decline in popularity (Thomas, 2010).

Joseph Gamgee in 1884 introduced an alternative surgical absorbent which consisted of an artificial antiseptic absorbent sponge composed of gauze, cotton and coconut fibre. A capsule of glass or gelatin containing antiseptic was placed in the centre ready to be released when broken (Gamgee, 1880).

Dressings made from foam were not introduced into wound management until the 1970s. The first product used in general wound management was Silastic Foam for cavity wounds. It was actually first used in 1962 as a diagnostic aid in detection of

sigmoid cancer (Cook and Margulis, 1962). The foam is formed in situ at the patient's bedside by mixing two liquid components. Later preformed foam membranes with and without adhesive coating were developed. These early dressings had relatively poor absorbency. Widespread acceptance began when products made from hydrophilic polyurethane were developed. Many were also bonded to a semipermeable polyurethane film or a second thin sheet of closed cell foam.

1.10 Allevyn dressing

Among the successful commercial foam dressing is Allevyn dressing marketed by Smith and Nephew company. The non-adhesive form was launched in 1987 and then the adhesive version in 1995. It is made of hydrophilic polyurethane prepolymers with greater affinity for aqueous solutions, in contrast to hydrophobic polyurethane foam used in Lyofoam. Allevyn dressing can absorb up to 10 times its own weight (Argirova et al., 2007)

It has been given a pseudoscientific name,'hydrocellular', which gives little information to most clinicians. Allevyn has undergone a number of modifications and developments to improve its performance as a dressing. For example, in order to provide a moist environment for wound healing, a more permeable outer film was introduced in 2006 to increase its fluid handling capacity. Allevyn is indicated in a number of different wound types and with moderate to high levels of exudates, including ulcers, surgical wounds, burns and donor sites. It is useful in reducing

hypergranulation when applied with slight pressure whilst maintaining a low adherence to facilitate easy and painless removal. Other advantages include its cushioning and pressure-protective effect, it insulates, and conforms well to body surfaces. Disadvantages include its relatively high cost and periwound skin maceration when compared to silicone type dressing (Woo et al., 2009).

1.11 Wound dressing research

The commonest wound dressing still widely used today for exudative wounds is saline-soaked gauze dressing, mainly due to its relatively low cost and simplicity. However, patients consistently experience more pain with gauze compared to other advanced moisture balance dressings such as foam and hydrocolloid dressings (Coutts et al., 2008, Viciano et al., 2000). Based on recent evidence, the efficacy of modern dressings over the saline-soaked gauze remains controversial (Chaby et al., 2007, Ubbink et al., 2008).

Efficacy is defined as the capacity for producing a desired result or effect. In the context of modern wound dressing, the efficacy consists of improvement in the wound, healing time and satisfying other claims made by the manufacturer. In wound dressing research, healing does not always have to equate to total wound closure because some chronic wounds take a long time to heal. Hence, outcomes such as wound size reduction, area of epithelization, and changes in wound conditions are used. The common parameters measured for wound conditions are presence of granulation tissue, necrotic tissue or slough, exudate level, and odour.

Other outcomes that have been measured include biomarkers (such as cytokines and growth factors), infection (such as appearance of wound and time to resolution), patients' symptoms and signs (such as pain and oedema), and dressing performance (such as adhesiveness and ease of removal).

An important factor that can adversely affect the healing process is pain. It has a negative impact on the patient psychologically and physically such as, the patient's mood, as well as, the patient's mobility and daily activities (Woo and Sibbald, 2008, Woo et al., 2008b).

Many studies have looked at the pain aspect associated with modern dressings. Pain experienced by patients can be generally divided into 2 instances: pain in between dressing changes and pain experienced during dressing changes. The highest level of pain was shown to be at the time of dressing change especially during its removal and wound cleansing (Woo et al., 2008a, Hollinworth and Collier, 2000, Kammerlander and Eberlein, 2002).

1.11.1 Randomized controlled trial

Since the middle of the 20th century, emphasis on evidence-based practice has increased. This refers to clinical decision making that is based on the best available evidence with practitioners reviewing information from powerful data, rather than relying on single observations or customs. Different types of evidence are available which have been organized into a hierarchy. At the top of the hierarchy is the meta-

analysis of several well-conducted randomized controlled trials (RCT). This is followed by the individual RCTs. For a wound care practitioner or physician, it is important to determine which dressing materials are best for patients where the main aim is healing and the absence of complications. One of the difficulties that researchers and clinicians face is the paucity of high-quality evidence from trials in wound management. The trials are often based on inadequate sample sizes, have short follow-up periods, non-random allocation to treatment groups, non-blinded assessment of outcomes, and poorly selected control groups and concurrent interventions.

RCTs are universally acknowledged as the study design of choice for comparing treatment effects as they eliminate several sources of bias. Nevertheless, randomization is not sufficient to ensure a study at low risk of bias. Trials should also ensure proper allocation concealment and blinding of outcome assessors, and use patient-oriented outcomes (Eskes et al., 2012). With this in mind, we have conducted a prospective multicentre single-blinded randomized controlled trial in humans to evaluate the efficacy of chitosan sponge against Allevyn dressing for exudative wounds.

2.0 Objectives of the study

2.1 General objectives

1) To evaluate the efficacy of locally produced chitosan sponge in comparison with a commercialized Allevyn® foam dressing in promoting wound healing.

2.2 Specific objectives

1) To compare the efficacy of Chitosan sponge with Allevyn® for the treatment of exudative wound in term of healing or epithelization rate.
2) To compare the efficacy of Chitosan sponge with Allevyn® for wound bed preparation in terms of formation of healthy granulation tissue and reduction of necrotic tissue.
3) To assess the practicality of both dressings in terms of adherence to wound bed, ease of removal, level of pain during removal, level of itchiness, periwound skin quality and exudate levels.
4) To determine the adverse reaction of the Chitosan sponge dressing.

2.3 Research hypothesis

There are significant differences in efficacy and healing properties between Chitosan sponge and Allevyn® as dressings for exudative wounds.

2.4 Null hypothesis

There is no significant difference in efficacy and healing properties between Chitosan sponge and Allevyn® as dressings for exudative wounds.

3.0 Material and methods

3.1 Study design

This was a prospective, multicenter, single-blinded, randomized controlled clinical trial. The study was simultaneously conducted at the following health care institutions:

1) Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan (HUSM)
2) Pusat Perubatan Universiti Kebangsaan Malaysia, Cheras, Wilayah Persekutuan (PPUKM)
3) Hospital Kuala Lumpur, Wilayah Persekutuan (HKL)

Ethical approvals were obtained from all three of the above institutions Research Ethics Committee. The respective references were as follows:

1) FWA Reg. No: 00007718; IRB Reg. No: 00004494 for HUSM
2) Project Code: FF-124-2013 for PPUKM
3) Reg. No: NMRR-12-1232-14187 (IIR) for HKL

3.1.1 Patient selection

All patients with exudative wounds that required wound bed preparation or surgical intervention were screened for the study. Participants were selected after fulfilling the inclusion and exclusion criteria set.

3.1.2 Inclusion criteria:

1) Patients with exudative wound such as surgical wounds, ulcerative wounds and partial thickness burns
2) Wound that is expected to heal within 30 days with or without surgical intervention
3) Patients who are able to give consent either by themselves or through their legal guardian
4) Patients age ≥18 years and ≤ 70 years.

3.1.3 Exclusion criteria:

1) Severely contaminated or untreated wound with clinical signs of infection

2) Chronic wound that is not expected to heal within 30 days such as pressure sores or venous ulcers
3) Deep ulcer or dead space that cannot maintain contact with the dressing
4) Highly exudative wound that is expected to saturate dressings within 48 hours
5) Patients with history of allergy to seafood
6) Patients with uncontrolled diabetes mellitus, on steroid medications or who are immunocompromised
7) Pregnancy
8) Patient with skin pathology – eczema etc

3.1.4 Consent for the study

Written informed consents were obtained from all patients after they were counselled and they understood the information sheet given. For patients who were unable to give consent for themselves, consent was obtained from their legal guardian.

3.1.5 Randomization

The patients were randomized into either the treatment groups with chitosan sponge dressing or control group with the commercially available Allevyn dressing. Randomization is conducted using web-based software at *www.randomization.com*.

The order of randomization was printed out and kept at the research office in HUSM as reference for the investigators of the study.

3.1.6 Sample size

The sample size was calculated based on two means formula for independent observations. The power of the study was taken at 90% and alpha (type one error) as 5%. The calculations were based on a similar study by Karlsson M. et al (Karlsson et al., 2014). A reasonable clinical improvement was assumed to be an increased in healing rate of 10%,

Sample size calculations:

Power	= 90%
Type 1 error (α)	= 0.05
Standard Deviation (σ)	= 13.2
Difference of mean (δ)	= 10

The sample size required for each study limb, N = 37

Assuming a 10% dropout rate, N = 41

Total number participants required for the study, N = 82

3.1.7 Chitosan sponge

Preparation of chitosan sponge was carried out at the Advanced Materials Research Centre (AMREC-SIRIM), Malaysia. Pharmaceutical grade chitosan powder with molecular weight (Mw) at 634 kDa and deacetylation degree (DD) at 89% was purchased from Master-Pack Nutriceuticals Sdn Bhd, Perak, Malaysia.

Chitosan was dissolved in 1% (v/v) acetic acid to prepare a 2% (w/v) chitosan solution. About 20% (w/w) glycerol were added as a plasticizer, followed by neutralization with sodium bicarbonate to achieve a pH of 6.2. The chitosan solution was frozen at −20◦C and lyophilized for 24 hours to create the porous structure. The chitosan sponge was sterilized using ethylene oxide (EO) according to the International Standards Organization (ISO) guidelines (Part 10993–7:1995: Ethylene Oxide Sterilization Residuals).

Chitosan sponge used for this study is a solid, yellow porous scaffold approximately 8 X 8 cm in dimensions (Figure 2). It is composed of water (94.6%), chitosan, acetic acid, glycerol and sodium hydrogen carbonate (5.4%).

3.1.8 Allevyn

Allevyn® (Smith & Nephew Medical, Hull, UK) is a type of foam dressing with a polyurethane contact layer that is highly absorbent and does not adhere to a full range of wound types. It is a type of bioactive wound dressing (Figure 3).
It is widely used around the world including Malaysia and is indicated for light to moderate exudative partial and full thickness wounds including ulcers, pressure

sores, surgical donor sites and debrided wounds, and partial thickness burns. Marked reduction in wound sizes and high satisfaction rates in terms of comfort and ease of application were reported with Allevyn dressings (Sylvia Leonard, 2009).

(a) (b)

Figure 2. Chitosan sponge (a) in sterile packaging (b) before application

(a) (b)

Figure 3. Allevyn dressing (a) in sterile packaging (b) before application