MODIFIED HALLOYSITE NANOTUBES AS CARRIER FOR ANTIMICROBIALS AGENT IN WOUND HEALING MATERIALS

BY

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A dissertation submitted in fulfilment of the requirement for the degree of Master of Science (Materials Engineering)

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May 2021

ABSTRACT

Plastic waste has become a significant issue globally, especially in the medical field, where most of the products are petroleum-based plastics. In this research, polysaccharide base films were utilized to overcome this issue. Polysaccharide materials such as thermoplastic sago starch (TPSS) promote wound healing; however, an infection is more likely to occur. Halloysite nanotubes (HNTs) are a biocompatible material that is used in medical applications. In this research, halloysite was modified by dispersing it with chloramphenicol solution. Chloramphenicol was loaded into halloysites using a magnetic stirrer. Thermoplastic sago starch (TPSS)/modified halloysite (MHNTs) biocomposite films were developed using the solution casting method. In addition, starch/ halloysite biocomposites films also were produced for comparison. Surface morphology characterization was done for the halloysite nanotubes, where it showed agglomerations of the modified halloysite. The scanning electron microscopy (SEM) images showed a good dispersion of the natural halloysite in the polymer matrix compared to the modified halloysite. Besides, modified halloysite shows higher agglomeration with further addition. X-ray diffraction spectra of the starch films showed an alteration with the addition of halloysite nanotubes, indicating intercalation of halloysites in the TPSS matrix. FTIR peaks of TPSS also showed changes with the addition of halloysite. The tensile properties demonstrated the mechanical properties of the halloysite/ thermoplastic sago starch. HNT and MHNT at 0.25 wt.% had slightly improved the tensile strength of the TPSS from 3.69 MPa to 4.11 and 3.76 MPa, respectively. However, the tensile strain was reduced when halloysite was introduced to the polymer matrix. The water absorption was decreased from 72.96% to 58.62% at 0.25 and 1 wt.% of HNT. Meanwhile, MHNT reduced the water absorption rate from 72.96% and 53.13% at 0.25 and 1 wt.% of MHNT, respectively. This reduction is attributed to its encapsulation of the chloramphenicol antibiotics which is a highly hydrophobic substance. Halloysite in nature is not an antimicrobial agent, and this was observed from the disc diffusion method. However, when HNT was modified using an antimicrobial substance, it successfully showed good susceptibility to bacterial culture. This indicates the ability of MHNTs to be used as an antibacterial carrier for wound dressing materials. The soil burial method was used to evaluate the biodegradability of TPSS biocomposites. MHNT has significantly reduced the biodegradability of TPSS compared to pure HNT. This leads to the fact that MHNT had good antimicrobial properties, which lead to the MHNT/TPSS to withstand the degradation from microbes in the soil. Results showed that halloysite could be a potential carrier for antibacterial agents. The thermoplastic sago starch can be an effective composite when incorporating with modified halloysite to withstand bacterial attacks. This research opens new doors for halloysite nanotubes to be further studied for carrying antibacterial substances or other drugs.

خلاصة البحث

أصبحت النفايات البلاستيكية مشكلة رئيسية في العالم، خاصة في المجال الطبي حيث معظم المنتجات عبارة عن مواد بلاستيكية قائمة على البترول. في هذا البحث تم استخدام مواد عديد السكاريد للتغلب على هذه المشكلة. تعمل مواد السكريات المتعددة مثل نشا الساغو البلاستيكي الحراري (TPSS) على تعزيز التئام الجروح ولكن من المرجح أن تحدث العدوى. تعد أنابيب هالوسايت النانوية (HNTs) متوافقة حيوياً بحيث تُستخدم في التطبيقات الطبية. في هذا البحث تم تعديل هالويسايت عن طريق اذابته بمحلول الكلورامفينيكول. تم مزج وادخال الكلورامفينيكول في في داخل انابيب الهالوسايت (HNTs) وعلى اسطحها باستخدام التحريك المغناطيسي. تم تطوير أفلام نشا الساجو البلاستيكي الحراري (TPSS) / والهالوسايت المعدل (MHNTs) باستخدام طريقة صب المحلول. بالإضافة إلى ذلك، تم إنتاج نشا الساجو البلاستيكي الحراري (TPSS) / والهالوسايت غير المعدل (HNTs) من أجل المقارنة. تم فحص سطح أنابيب الهالوسايت النانوية بالمايكروسكوب، حيث تُظهر تكتلات وتجمعات من هالويسيت المعدل. بالإضافة إلى ذلك، أظهرت صور الفحص المجهري الإلكتروني (SEM) توزيعا جيدا للهالوسايت في سطح البوليمر مقارنةً بالهالوسايت المعدل حيث يُظهر تكتلاً أعلى عند زيادته. أظهرت أطياف الأشعة السينية لأغشية النشا تغييراً مع إضافة أنابيب الهالوسايت النانوية وهذا يشير وجود الهالوسايت في سطح مركب البولمر TPSS. كما أظهرت قمم FTIR لـ TPSS تغييرات مع إضافة الهالوسايت (HNT). أظهرت خصائص الشد الخواص الميكانيكية لنشا الساجو / اللدائن الحرارية. حسنت HNT و MHNT عند 0.25% بشكل طفيف قوة الشد لـ TPSS من 3.69 ميجا باسكال إلى 4.11 و 3.76 ميجا باسكال على التوالي. ومع ذلك ، قل إجهاد الشد عندما تم إدخال هالويسايت إلى مصفوفة البوليمر. تم تقليل امتصاص الماء من 72.96٪ إلى 58.62٪ عند 0.25 و 1 ٪ من HNT. وفي الوقت نفسه ، خفضت MHNT معدل امتصاص الماء من 72.96٪ و 53.13٪ عند 0.25 و 1٪ من MHNT على التوالي. عندما يتم مزج HNTs في مصفوفة TPSS فإنها تقلل من امتصاص الماء. ومع ذلك، فإن إضافة MHNT قد قللت أيضًا من امتصاص الماء أكثر من HNT. وهذا يُعزى إلى تغليفه بمضادات الكلورامفينيكول الحيوية وهي مادة شديدة الكراهية للماء. هالويسايت في الطبيعة ليس عاملًا مضادًا للميكروبات وقد لوحظ هذا من فحص البكتيريا الذي تم اجراؤه. ومع ذلك، عندما تم تعديل الهالوسايت باستخدام مادة مضادة للميكروبات، فقد أظهر بنجاح قابلية جيدة للبيئة البكتيرية. هذا يشير إلى قدرة MHNTs على استخدامها كناقل مضاد للجراثيم لمواد تضميد الجروح. تم استخدام طريقة الدفن في التربة لتقييم قابلية التحلل البيولوجي للمركبات الحيوية TPSS وقد قلل MHNT بشكل كبير من قابلية التحلل البيولوجي لـ TPSS مقارنة بـ HNT النقي. هذا يؤدي إلى حقيقة أن MHNT له خصائص جيدة مضادة للميكروبات والتي تؤدي إلى / MHNT TPSS لتحمل التحلل من الميكروبات في التربة. أظهرت النتائج أن الهالويست يمكن أن يكون ناقلًا محتملاً للعوامل المضادة للبكتيريا ويمكن أن تكون لدائن نشا الساغو الحرارية مركبًا فعالًا عند دمجها مع هالويسايت المعدل لمقاومة الهجمات البكتيرية. يفتح هذا البحث أبوابًا جديدة لأنابيب هالويسيت النانوية لمزيد من الدراسة لحمل مادة مضادة للبكتيريا أو أدوية أخرى.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science (Materials Engineering)

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except were otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nader M.I Abuhamed

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ACKNOWLEDGEMENTS

Praise be to Allah, the most merciful and most compassionate, for his blessing and for giving me the opportunity, strength, and time to complete my dissertation.

My utmost pleasure to dedicate this work to my dear parents and my family, who granted me the gift of their unwavering belief in my ability to accomplish this goal: thank you for your support and patience.

A special thanks to my supervisor, Assoc. Prof. Dr. Ir. Zuraida Ahmad for her continuous support, encouragement, guidance, and leadership, and for that, I will be forever grateful. I would also like to thank my co-supervisor, Dr. Norshahida Binti Sarifuddin, for her support, guidance, and confidence in me, which inspired me to finish my master's degree.

I want to express my deep appreciation to all the technical staff in laboratories and the Department of Manufacturing and Materials Engineering staff for their assistance and cooperation throughout my research period. And I would also like to thank the staff in the postgraduate office and the department coordinator Assoc. Prof. Mohd. Hanafi bin Ani and all the administrative officers for their assistance and guidance throughout my studies.

I wish to express my appreciation and thanks to those who provided their time, effort, and support for this project. To the members of my dissertation committee, thank you for sticking with me.

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LIST OF ABBREVIATIONS

C	Collagen
CCSM	Chitosan-Collagen-Starch Membrane
cm	Centimeter
Cm3	Centimeter cubic
e.g.	exempli gratia
ECM	Extracellular Matrix
Et al.	Et Alia
etc.	Et cetera
FE-SEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier-Transform Infrared Spectroscopy
G	Glutaraldehyde
g	Gram
GAGs	Glycosaminoglycan
GPa	Giga pascal
HA	Hyaluronic Acid
HNT	Halloysite Nanotubes
ml	Millimeter
MHNT	Modified Halloysite Nanotubes
MIC	Minimum Inhibitory Concentrations
Min	Minute
µm	micrometer
MPa	Mega pascal
N	Newton
NaCl	Sodium chloride
Nm	Nanometer
PLLA	poly-L-lactic acid
PVA	Polyvinyl Alcohol
rpm	Revelation per minute
SEM	Scanning Electron Microscope
SG	Sago Starch
SY	soya protein
TEM	Transmission Electron Microscopy
TPSS	Thermo Plastic Sago Starch
WHO	World Health Organization
Wt.%	Weight percent
XRD	X-Ray Diffraction

LIST OF SYMBOLS

%	Percentage
W	Weight
W_a	Water absorption
ρ	Density
m_a	Mass after
m_b	Mass before
L	Length
W_m	Moisture content
b	Width
A	Area
d	Depth
ε	Strain
σ	Stress
t	Thickness

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Wound and injuries had always been one of the significant problems for public health. The world health organization (WHO) has announced in 2000 that around 5 million people died from injuries. Moreover, in the same year, Malaysia had 13,401 injury cases, and it was estimated that 440 cases were deadly (Hasni, Junainah, & Jamaliah, 2003). In addition to mortality, the formation and healing process of wounds is a minor problem. However still, it is considered a big issue because it might lead to other major health problems. The number of injuries that occur every year worldwide are countless due to surgical procedures, ulcers, and burns that do not heal. A wound is a physical injury that usually damages the underlying tissues, and it is considered one of the most extensive clinical challenges in the world (Merriam & Webster. 2015). Some wounds like foot and pressure ulcers occur because of complications of some diseases such as diabetes, and these wounds are called chronic wounds. There is another type of wound called acute wounds, which are caused by skin injuries. These wounds are less harmful than chronic wounds because they can be healed more easily (Hasni, Junainah, & Jamaliah, 2003). Throughout history, several wound healing methods have been used to improve the healing process of wounds. Although wounds can heal naturally on their own, some factors delay wound healing, such as bacterial infections or exudate production at the wound surface. Therefore, the treatment and management of wounds are essential aspects to avoid the mentioned problems. Moreover, the market value for care products used to treat wounds was estimated at 62.6 billion MYR in 2010, reflecting an increase in global clinical demand for those products (Sen et al., 2009).

The dressing provides wounds with the right environment under certain pathological conditions that make them heal at a maximum rate while giving a cosmetically acceptable appearance; they protect the wound from external influences and keep them moist to ensure proper healing. Modern dressings absorb wound exudate to control the healing process. Interestingly, to improve the absorption of wound exudates, dressings should be incorporated with a bioactive material such as polysaccharides (Sweeney, Miraftab & Collyer, 2012). It is important to note that wound treatment interventions like dressings could support and accelerate the healing process. Hence, selecting the right wound healing materials by considering the healing ability and antimicrobial activity are essential aspects of dressing innovation. Polysaccharides are biologically derived polymers that had been extensively used as wound healing dressing material. And this is because of their availability, versatility, biocompatibility, and antimicrobial properties (Chen & Chang, 2008). Chitin, cellulose, starch, and glycogen are types of polysaccharide which have been extensively reported to be a suitable material in the medical industry, thus in this project, the sago starch (SG) polysaccharide is proposed as a potential candidate for fabricating of the films.

On the other hand, halloysite nanotubes (HNT) would help increase the mechanical properties and be an effective candidate as antimicrobial agents in wound healing application. HNT has a hollow structure, which has a tunable surface property and capable of encasing antiseptics. One of the most practical features of HNT is that they do not require expensive and high energy-consuming processes of exfoliation. Several reports were devoted to producing polymeric films and membranes containing antibacterial compounds incorporated with HNT (Morrison, Misra & Williams. 2016).

Therefore, the preparation and characterization of thermoplastic sago starch- halloysite nanotube composites, as wound healing application, will be presented in the later section.

1.2 STATEMENT OF THE PROBLEM

Polymers are materials that are suitable for all medical applications due to their vast and varied properties. In the past decade, plastic waste has become a significant issue globally, especially in the medical field, where most of the products are petroleum-based plastics. These plastics are harmful to people and the environment because petroleum plastics are non-biodegradable and very damaging. An appropriate wound dressing material should extract exudates and toxic substances from the surfaces of the wound, facilitate gaseous exchange, protect the wound from bacterial infection, retain high moisture at the wound/dressing site, and it should be removed safely without damaging the wound (Dhivya, Padma & Santhini, 2015). Many researchers have adopted the use of biopolymers since there are more environmentally friendly, and most importantly, biocompatible many in the medical application (Waghmare et al., 2018; Adeli, Khorasani & Parvazinia, 2018; Hassan et al., 2017; Baghaie et al., 2017; Amal et al.,2015). Films are the most ideal for wound dressing, and biopolymers-based material would be used as a based material because of its non-toxicity and biocompatibility compare to synthetic polymers. Polysaccharide polymers such as thermoplastic sago starch (TPSS) films have been successfully developed at IIUM (Ahmad, Hermain & Abdul Razak, 2015; Ahmad, Hermain & Abdul Razak, 2018). Besides, researchers have proven polysaccharides starches to be valid materials for wound dressing applications (Mandal et al., 2014; Amal et al., 2015). Polysaccharides wound dressing materials such as starch promote and speed up the healing process during the wound healing process. However, starch hydrophilicity absorbs the exudates from the wounds during the healing process, which causes most likely promotes a bacterial infection that slows the healing process. Therefore, an antimicrobial agent needs to be used to overcome this issue (Mandal et al., 2014; Ramnath et al., 2012).

Halloysite nanotubes (HNT) have been used widely in drug delivery and tissue engineering scaffolds in the medical field. Halloysite does not have any antibacterial effect, and many studies reported that HNT needs to be modified to have the antibacterial impact (Kurczewska et al., 2017; Makaremi et al., 2017; Biddeci et al., 2016). In this research, modified HNT is proposed as the antibacterial filler for the wound dressing film. The advantage of the halloysite as the antibacterial carrier or filler is due to its hollow structure. Therefore, besides the HNT is reported to have a good impact on polymer composites' mechanical and thermal properties with a little bit of modification, it is expected to give suitable antimicrobial activities.

1.3 RESEARCH OBJECTIVES

The study aims to develop sago starch (SG) wound dressing incorporated with Halloysite nanotubes (HNT) as a carrier for antimicrobial agents for wound healing. And to obtain this aim, several objectives need to be achieved:

1- To characterize the microstructure of halloysite nanotubes and TPSS / HNT nanocomposites. In addition to identifying the functional groups and crystallinity of the nanocomposites.

2- To evaluate the effect of unmodified halloysite nanotube and modified halloysite content on the physical (water absorption), mechanical (tensile), and degradability properties of the TPSS/HNT nanocomposite films.

3- To investigate the antimicrobial properties of TPSS/HNT nanocomposite using Kirby-Bauer disk diffusion method.

1.4 SIGNIFICANCE OF RESEARCH

This research introduced a wound dressing developed from biocompatible, nontoxic, inexpensive, widely available, and biodegradable resources. The produced biocomposite was made from sago starch, halloysite nanotubes, distilled water, and plasticizer glycerol in casted films. This developed biocomposite reduced the dependency on synthetic polymer materials used in the wound dressing industry since that waste is harmful to the environment and costly to damp it. Sago starch is widely available in malaysia. With this new era of advanced biomaterial to replace synthetic polymers, it opened many doors in the research industry to utilize such materials for medical application. Halloysite is a well-known material used in drug delivery and many medical applications. In this research, tuned and modified halloysite opened new doors for wound dressing applications, especially with biocompatible sago starch.

1.5 SCOPE OF THE STUDY

This scope of the research focuses on the development and preparation of biocompatible wound dressing films of sago starch filled with halloysite nanotubes (HNT). This research evaluated the effect of modified halloysite nanotubes content on the sago starch at different loadings. HNT was modified by mixing it with antibiotics in a magnetic stirrer until completely dissolved. A comparative study compared the addition of unmodified HNT & modified HNT to thermoplastic sago starch and their effects on antimicrobial activities, chemical bonding, crystallinity, biodegradability, microstructure, physical and mechanical properties of the wound dressing films.

1.6 DISSERTATION ORGANIZATION

Five chapters organize this research work. Chapter one introduces the wound dressings and the environmental and health issues that could result from it and ways to overcome these problems; it also shows the objectives of this research and the significance and scope of this study. Chapter two is a theoretical description of the sago starch, halloysite nanotubes, and reviews of the recent literature of their uses in the medical field. Chapter three describes comprehensively the experimental setup and work procedures that are adopted by this research. It also discusses the work fabrication and experimental works to produce the biocomposite films and characterization method to evaluate the properties of wound dressing material. Subsequently, chapter four discusses and evaluates thoroughly the results obtained from experimental work. Finally, chapter five presented a general conclusion and recommendation for further research based on the findings from this work.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

A suitable wound dressing material should extract exudates and toxic substances from the surfaces of the wound, facilitate gaseous exchange, protect the wound from bacterial infection, retain high moisture at the wound/dressing site, and it should be removed safely without damaging the wound. This chapter will extensively explain the type of wounds, wound healing process, the polysaccharides material, and the reason to be a suitable candidate for medical application, especially in wound dressing scaffold, membranes, and films. Starch is a type of polysaccharide, and this section will have an explained review on the structure and properties of starch and what research had reported about starch and its uses in the medical field. Many scholars have extensively studied halloysite nanotubes, so a review about halloysite nanotubes' structure, properties, and applications are also covered.

2.2 TYPE OF WOUNDS

Generally, wounds can are classified into two major types such as acute and chronic, as shown in Figure 2.1. Acute wound involving both the epidermis and superficial dermis, or full-thickness in which the subcutaneous layer is compromised (Driefke, Jayasuriya, & Jayasuriya, 2015). Conversely, chronic wounds occurred due to the complications of other disease processes such as foot ulcers from diabetes, pressure ulcers resulting from spinal cord injuries, and fungating or malignant wounds. The details of each classification are described in the sub-section below

In general, wounds can be categorized into two main sections acute and chronic, as shown in Figure 2.1

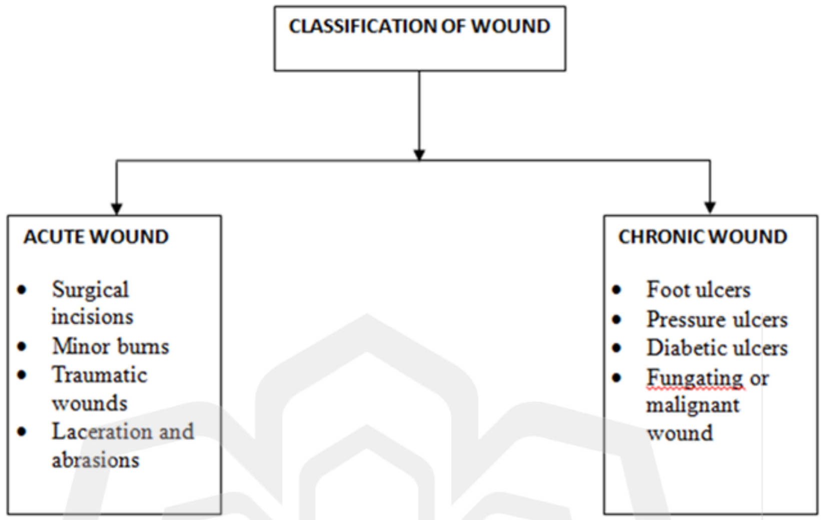

Figure 2.1: Wound classification

2.2.1 Chronic Wounds

Chronic wounds are characterized by cellular aging, recurrent infections, and delayed healing (Schultz et al., 2011). There are differences between acute wounds and chronic wounds. The wound healing process tends to heal acute wounds after 3 weeks, and chronic wounds take at least 3 months to continue. The healing of wounds, including acute and chronic wounds, is one of the significant clinical challenges of the world.

2.2.2 Acute Wounds

Minor burns, surgical incisions, and some traumatic injuries are examples of acute wounds with significant complications associated with each injury. In this research, the focus will be on acute wounds mostly. A critical wound is damage that occurs on the skin. The main difference between an acute wound and a chronic wound is that the acute