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MIGRAINE: PATHOFYSIOLOGICAL MECHANISMS

LUCIEN ENGELEN: MAJOR INNOVATIONS IN HEALTHCARE

DYSTONIA AND PSYCHIATRIC DISORDERS: CONNECTED?

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PREFACE FROM THE EDITORIAL BOARD

In this constantly changing world it may be hard to keep up with reality. If you just think about the fact that you can check your emails or Whatsapp messages anywhere and anytime, while your parents had never even heard of internet while growing up and your grandparents probably did not even have access to a telephone, you can only imagine what will happen in the coming decades. You have probably heard about upcoming innovations such as smart watches and glasses, drones delivering stuff to your house and self-parking cars, but did you know there are devices that can be charged wirelessly? Or that there are artificial eyes that can help blind people see again?

All these changes start with science. Science helps people to further understand processes and to use this knowledge to develop better tools and devices to make life better, more bearable or just more fun. In this edition there are several articles which clearly demonstrate this process. For example, in one scientific article the authors look for a better composition of substances to use in wound dressings, in order to develop a material to increase wound healing. Interestingly, the individual compounds of their new formula have been previously studied, but never before in this combination. Furthermore, we present research done in our own Radboudumc that is developing a smart 'health patch' that registers different parameters of the condition of the body simply by being adhered to the skin. This patch would make it easier for healthcare professionals to monitor patients, for instance after major surgery. In this edition there is an interview with the PhD-student that studies the applicability and usability of the health patch, as well as a short explanation of how the health patch works. Also, you will find an interview with Lucien Engelen, director of REshape Center Radboudumc, who is one of the most important promotors of healthcare innovation. He gives his opinion on the developments in healthcare that will take place in the near future, which our current readers will likely be confronted with on a daily basis.

In addition to these articles clearly focusing on innovation, you will find two other interesting scientific articles that also share a common subject: the brain. The first article provides a clear overview of the current knowledge of the pathogenesis of migraine, one of the most common diseases among younger people. The authors of the second article studied the relationship between dystonia and psychiatric disorders. Not only do they focus on the identification of a relationship between these disorders, but they also formulate a hypothesis based on the shared involvement of different neurotransmitter pathways including serotonin and dopamine.
All of the articles that are published, not only by RAMS, but in all scientific journals, contribute to the development of new tools that are useful in healthcare or daily activities. You too can contribute to the future. Will your article be published in our next edition?

David Wolthuis
Editor-in-Chief

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MIGRAINE: A COMMON DISEASE WITH POSSIBLE DISASTROUS CONSEQUENCES

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ABSTRACT:

BACKGROUND: The World Health Organization (WHO) established a worldwide change on the view of migraine by stating it to be one of the four most disabling chronic medical disorders. Headache disorders bring along personal suffering, impaired quality of life and financial costs. There are two forms of migraine, migraine with aura (MA) and migraine without aura (MO). In 1975 the first correlation between migraine and ischemic stroke (IS) was observed with a twofold higher risk of developing IS in migrainous patients.
OBJECTIVE: The aim of this article is to summarize the known information of the mechanisms behind migraine. The well-described mechanisms behind migraine and stroke will lead to the development of future diagnostics and therapeutics to decrease the prevalence of migraine and strokes.
RESULTS: Migraine is caused by cortical spreading depression (CSD) which is a wave of intense nerve cell activity that spreads through an unusually large area of the cortex. This hyperexcitable phase, that may be caused by local elevations of extracellular potassium, is followed by a wave of neuronal inhibition. Inhibited neuronal cells require less oxygen and therefore cerebral blood flow decreases. The trigeminal nerve system carries nerve signals from meninges and these blood vessels and establishes pain sensation. Three possible pathophysiological mechanisms, by which migraine may induce a stroke, can be explained: cortical spreading depression, spasms of the large intracerebral arteries and hypercoagulability related to vascular endothelium dysfunction.
CONCLUSION: Future research on diagnostic and therapeutic tools on possible targets in these mechanisms has to be done to decrease the prevalence of migraine and eventually stroke caused by migraine.

WHAT IS KNOWN? The WHO states that migraine is one of the four most disabling chronic medical disorders.

WHAT IS NEW? An overview of the current literature containing the possible mechanisms, by which a stroke can be induced by migraine; cortical spreading depression, spasms of the large intracerebral arteries and hypercoagulability related to vascular endothelium dysfunction.

KEYWORDS: Migraine, Stroke, Cortical spreading depression

Introduction

Migraine still remains to be one of the most misunderstood, poorly recognized and mistreated medical disorders. These headaches have been considered imaginary for a long time and are only since a few decades recognized by physicians as a disease. Patients still believe physicians are skeptical towards migraine and therefore seek no medical consolation for their pain; only 30% of the patients suffering from migraine visits a PD in the Netherlands[1]. A report published by the World Health Organization established a worldwide change on the view of migraine by stating it to be one of the four most disabling chronic medical disorders[2]. In the past year 47% of the world population has had a headache of which 10% had migraine. Headache disorders bring along a great burden of disease including personal suffering, impaired quality of life and financial costs[3]. Many triggers are registered that could evoke an attack such as emotional stress or physical activity. Migraine arises from a dysfunction in the nervous system and most likely from the brainstem. There are two forms of migraine. One, migraine with aura (MA), involves sensory hallucinations which are not present in the other form, migraine without aura (MO). Aura often precedes an attack[2]. Migraine presents itself with a great interindividual variety in frequency, duration and experience of the attack. Current research into migraine is focused on the involvement of genetics, imaging of the brain and molecular biology.

Migraine-related ischemia

Ischemic stroke (IS) is a cerebrovascular disease, often associated with migraine. The prevalence of IS is 1/500 in patients above the age of 65 years[4]. In 1975 the first correlation between migraine and IS was observed with a twofold higher risk of developing IS in migrainous patients. Studies including MRI brain imaging found an increase of IS in the posterior circulation territory in migraine patients. Furthermore, women with migraine have a higher risk of developing white matter lesions[5].
The association between migraine and stroke has proven much more complex than initially recognized, especially because a number of vascular disorders can cause both MA and IS. Moreover, cerebral ischemia can trigger MA. Epidemiological studies have reported an association of migraine (mostly with aura) with ischemic stroke, hemorrhagic stroke, coronary events, and mortality[6,7]. Migraine can directly lead to a stroke, which is called a migrainous infarction, or an indirect relation exists when a stroke occurs during the time between migraine attacks. Therefore, it is of importance to further clarify the underlying mechanism of migraine to be able to identify a mechanism by which migraine increases the risk of getting a stroke. Finally, the discovered mechanism between migraine and stroke will lead to the development of future diagnostics and therapeutics to decrease the prevalence of migraine and also strokes[8]. This review is constructed of literature on underlying mechanisms causing migraine and the association with cerebral stroke.

The inclusion for articles was based on impact factor, the use of English language, primary or secondary publications, number of times the article was cited, date of release and relevance.

Pathophysiological mechanism of migraine

To elucidate the possible mechanisms of migraine causing stroke, it is important to have a clear idea of the mechanisms underlying migraine. This disorder has an extensive history of hypothetical mechanisms containing vascular causes and a dysfunction of the trigeminal nerve. The most acknowledged hypotheses and how they can cause the typical migraine symptoms is elaborated below.

Cause of aura

Aura is caused by cortical spreading depression (CSD). CSD is a wave of intense nerve cell activity that spreads through an unusual large area of the cortex, especially the occipital cortex which controls vision. This hyperexcitable phase is followed by a wave of neuronal inhibition that can be seen on fMRI or EEG. During this phase of inhibition the neurons are non-excitable. Patients experience bright hallucinations, which can be explained by the excited state of neurons, followed by dark spots, which can be subscribed to the inhibition of neurons. When neurons are excited the need for oxygen increases which reflects in an increased cerebral blood flow (CBF). In the inhibited phase the demand for oxygen decreases, explaining the CBF changes. The electrical waves move extremely slowly: two to three millimeters a minute while normal nerve action potentials travel with speeds of 120 meters per second. This rate of spread probably explains why the aura is seen first, before the actual pain starts to set in. The posterior parts of the brain, primarily the occipital lobe, are initially affected. When the spread has reached the central sulcus, the pain starts to set in[2].

Trigeminovascular nociception

The pain during a headache is established by a network of nerves, called the trigeminal nerve system, which carry nerve signals from meninges and blood vessels. These nerves signal to the trigeminal nucleus in the brainstem and from there the signal is conveyed up through the thalamus to the sensory cortex. Which mechanism activates the trigeminal nerve is under debate. The first hypothesis states that CSD activates this pathway directly via a wave of increased neurotransmitter and ion release. Patients without aura have a CSD activation that is present on sites of the cortex or on subcortical regions that do not produce recognizable symptoms. Other researchers claim that the genesis of pain is placed in the brainstem. There is evidence that three nuclei, the locus coeruleus, raphe nucleus and periaqueductal gray, in the brainstem are active during and after a migraine attack. These nuclei could fail in inhibiting signals from trigeminal nerves as they are oversensitive to signals and can even send pain signals without any input[8].

Migraine considered as a channelopathy

Three gene mutations (in the CACNA1A, SCN1A and ATP1A2 genes) are associated with migraine. These genes encode neuronal ion channels and pumps. Mutation in those genes could cause channelopathy which can be responsible for getting migraine. It is believed that CSD is caused by local elevations of extracellular potassium [K+] which causes a decrease in K+ gradient, consequently causing a more positive membrane rest potential . Small changes in current can trigger an action potential and in this way get the cell in a hyperexcitable state[9] . The level of cortical excitability, which modulates the susceptibility to CSD, varies within the population. The level of excitability is low in people without migraine and high in patients with MA[8].

Pathophysiological mechanisms of migraine causing stroke

Three possible pathophysiological mechanisms, by which migraine may induce a stroke, can be explained: cortical spreading depression, spasms of the large intracerebral arteries and hypercoagulability related to vascular endothelium dysfunction[10]. These are the most fundamental mechanisms with sufficient evidence supporting these theories and they are elaborated below.

Cortical spreading depression

The mechanisms by which CSD may cause stroke remain purely hypothetical. In patients with migraine, inhibition of neurons can decrease CBF. This reduced flow can be decreased to such a low rate that dangerous ischemic levels could be reached with stroke as an effect. Currently, the medication that is given to patients with migraine have a rapid effect on arresting migraine attacks. These medicines work as a vasoconstrictor on the cerebral blood vessels and increase the risk of stroke, because it makes the chance of blockage of the vessels more prominent. Another theory described in this study is that a low threshold for CSD, that characterizes the brain in MA, also lowers the threshold for cerebral ischemia. There is some evidence for this theory that has been gathered by doing experiments on mice, but it remains controversial if this is plausible[8].

Vasospasm

In the past, before the mechanism of CSD was known, migraine was thought to be a vascular disease. For this reason the found literature underlying vasospasm is often outdated. Vasospasm, one of the possible mechanisms by which migraine can cause a stroke, is related to the vascularity of the brain. This theory suggests that by vasoconstriction of the intracerebral arteries, ischemia in the affected region and aura in the patient occurs[11]. A response of hyperaemia follows, leading to vasodilatation and headaches: a common symptom of migraine. Another dated article written by Cole et al.[12] assumes that during a migraine attack the cerebral blood flow diminishes, resulting in ischemia in the cells of the vasa vasorum (smaller blood vessels that perfuses the walls of the bigger blood vessel). This could develop necrosis of the endothelial cells and possible rupture of the cell wall when perfusion is restored. In migrainous cases, these patients show different possible causes of vasospasm. Vasospasm may have an underlying cause not related to migraine which will be disregarded as it is not a point of interest in this review. Another possible mechanism in which migraine is related, is that drugs which are given to migrainous patients can provoke vasospasm, such as ergotamine. The outbreak of arterial vasospasm can be due to substances as endothelin and serotonin. Although the distribution of vasospasm does not correlate with the affected area of the neurological effects of a migraine attack, it can be suggested that vasospasm is a side effect of migraine and it can be seen when a migrainous infarction occurs[9,12].

Hypercoagulability

Different studies show an increased production of platelet activating factor (PAF) during a migraine attack in MO patients. The sources of this PAF production are the endothelial cells in the brain and the mast cells[13]. PAF can trigger thrombotic cascades, as can be seen in figure 1, and is one of the most important molecules in causing hypercoagulability. There are several mechanisms correlating migraine with cerebral ischemia via hypercoagulability. Firstly, the activation of PAF causes accumulation of platelets to stop the bleeding when endothelial cells are damaged. When platelets are overactivated, it may result in ischemic stroke[14]. Secondly, PAF causes the release of von Willebrand factor (vWF), which is an important protein in coagulation. The primary function of

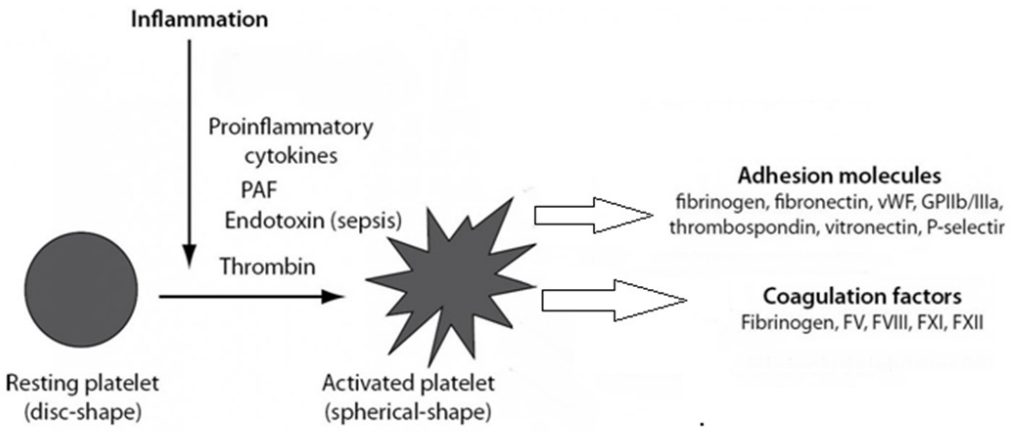

Figure 1 *Schedule with different proteins which are involved in inflammation and platelet activation*

vWF is to bind other proteins involved in blood clotting, in particular factor VIII, which is an important platelet adhesion molecule. A small study in people with migraine has shown a release of vWF during migraine[15]. This study did not examine whether this can also lead to an increase in hypercoagulability in migraine patients. Inflammation can be caused by platelets for the following reasons. Firstly, in a study from Taffi R et al., migraine patients show a significantly greater production of peroxynitrite (ONOO-) in their platelets compared to the production of ONOO in the platelets of controls. Furthermore, patients showed an increased expression of inducible Nitric Oxide Synthase (iNOS) in comparison to control subjects. iNOS can react to peroxynitrite which is a marker for oxidative stress[16]. Oxidative stress can cause endothelial cell death and results in inflammation by activating the PAF pathway. This inflammation can lead to more hypercoagulability via platelet activation. The platelet activation causes an increase in many different proteins and molecules like adhesion molecules and coagulation factors. A higher coagulability can cause blood clots which can block an artery and cause stroke.

Discussion

Although there is a substantial amount of research on migraine and stroke, the studies used in this review had some limitations. A lot of studies that were done examined different proteins and whether the concentrations of these proteins were elevated in migraine patients compared to controls. Unfortunately, the relationship between the different proteins was not well defined in these studies. Moreover, no evidence was found for the correlation between the elevation of these proteins and the onset of stroke. The mechanism underlying migraine is just a hypothesis, so the mechanism which describes the relation between migraine and stroke can only be postulated in the following plausible summarized theories. Cortical spreading depression is one of the underlying mechanism how stroke can be caused by migraine. During CSD the blood flow decreases and can even reach ischemic levels, so a stroke can occur. On the other hand, controversy remains over the actual establishment of pain in CSD. The underlying mechanism of CSD and pain is therefore still questionable. A possible way in which vasospasm can provoke a stroke can be explained according to the vascular theory. This theory asserts that vasoconstriction of the intracerebral arteries during a migrainous attack cause ischemia in that specific area which can result in a stroke. The second pathway of a stroke inducing mechanism is in the cells of the vasa vasorum. Ischemia can result in necrosis of the vasa vasorum and possibly rupture of the entire blood vessel wall when perfusion is restored. When endothelial cells are damaged, PAF activation leads to accumulation of platelets. It is well known that overactivation of platelets can cause stroke. Also PAF induces the release of the von Willebrand factor which leads to blood clotting. There is also an increase in production of peroxynitrite and induced nitric oxide synthase, which are both markers for oxidative stress. After oxidative stress development cell death may ensue, which can cause inflammation. Inflammation can be a source of hypercoagulability. Blood clotting can be an important cause of strokes, because it can block the vessels, which could have cerebral ischemia as an effect.

Recommendations

Several mechanisms and proteins are involved in migraine causing a stroke. Future research on diagnostic measures and therapeutic interventions on these possible mechanisms has to be done to decrease the prevalence of migraine and eventually stroke caused by migraine. Several targets for therapeutic and diagnostic measures can be imagined based on the three mechanisms: CSD, spasms of the large intracerebral arteries and hypercoagulability related to vascular endothelium dysfunction. First of all, there could be a therapy for the CSD K+ ion-channel dysfunction, which is hard to target specifically. A possibility of a future diagnostic tool is to do genetic tests based on the three genetic mutations that encode the potassium channels and pumps. Patients with the genetic defect could get preventive stroke therapy or screened for other risk factors for stroke. During CSD a critical level of CBF can be reached what may result in cerebral ischemia. A population at risk in migraine patients could be formed which would include patients with a cardiovascular background, obesity or a reduced vascular plasticity for example. With this information preventive measures could be initiated to prevent strokes. Currently, medication is given to patients to immediately alleviate the pain symptoms. The medication, that has vasoconstrictor properties, should be used as little as possible as it increases the risk of stroke. Migraine patients have temporary lesions in the brain, a transient ischemic attack (TIA), which can be visualized by MRI[10]. These lesions are an indicator for having an increased chance of the development of stroke. Possibly, patients with suspected migraine can undergo a MRI-scan to confirm having migraine-related lesions and thus a higher stroke risk. A preventive therapy against migraine can be given to these early-diagnosed patients, which hopefully can prevent occurrence of a stroke. Unfortunately, this preventive therapy has not yet been developed, so further research on this therapy has to been done. Targeting vasospasm is hard because the exact mechanism of how vasospasms occur is not well known. It is not recommended to give vasoconstrictors or vasodilators because they respectively increase the risk of stroke or worsen the symptoms. Stroke caused by hypercoagulability could be reduced by providing patients with blood thinners. This hypothesis is based on the fact that blood that is thinned flows faster and has a lower probability

to clot. Because the risk of developing a blood clot is less prominent it is more unlikely to cause a stroke. These targets are hypothetical based on the mechanisms that are found and explained in this review. They all could be possible indications for further investigation. These investigations will hopefully give insight into this complicated and underdiagnosed disorder and prevent disastrous consequences.

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References

1. MJJC P. Hoe vaak komt migraine voor en neemt dit toe of af? 2014; Available from: http://www.nationaalkompas.nl/gezondheid-enziekte/ziekten-en-aandoeningen/zenuwstelsel-enzintuigen/migraine/trend/.
2. Dodick DW. Why Migraines Strike. Scientific American. 2008.
3. WHO. Headache disorders. 2012; Available from: http://www.who.int/mediacentre/factsheets/fs277/en/.
4. Vaartjes I. Hoe vaak komt een beroerte voor en hoeveel mensen sterven eraan? 2014; Available from: http://www.nationaalkompas.nl/gezondheidenziekte/ziektenenaandoeningen/hartvaatstelsel/beroerte/omvang/.
5. Guidetti D. Migraine and Stroke: "Vascular" Comorbidity. Frontiers in Neurology. 2014.
6. Bigal ME. Migraine and cardiovascular disease: a population-based study. Neurology. 2010.
7. Sacco S. Migraine and hemorrhagic stroke: a meta-analysis. Stroke. 2013.
8. Kurth T. Migraine and stroke: a complex association with clinical implications. The Lancet Neurology. 2012.)
9. Pietrobon D. Pathophysiology of migraine. Annual review of physiology. 2013
10. Pezzini A. The Migraine-Ischemic Stroke Connection: Potential Pathogenic Mechanisms. Current molecular medicine. 2009.
11. Wolf ME. Clinical and MRI characteristics of acute migrainous infarction. Neurology. 2011.
12. Cole AJ. Migraine With Vasospasm and Delayed Intracerebral Hemorrhage. Archives of neurology. 1990.
13. Sarchielli P. Platelet-activating factor (PAF) in internal jugular venous blood of migraine without aura patients assessed during migraine attacks. Cephalalgia. 2004.
14. Arboix A. Ischemic Stroke as First Manifestation of Essential Thrombocythemia. Stroke. 1995
15. Cesar JM. Increased levels of plasma von Willebrand factor in migraine crisis. Acta neurologica Scandinavica. 1995.
16. Taffi R. Platelet membrane fluidity and peroxynitritelevels in migraine patients during headache-free periods. Cephalalgia. 2005.

STUDENTS 4 BEST EVIDENCE

By Kim Cortenbach

Currently, not only is RAMS doing its best to enthuse students about research, but Students 4 Best Evidence (S4BE) is doing the same on a worldwide scale. The main aim of S4BE is to promote an interest in evidence-based decision-making amongst young people, but with a particular focus on student health professionals. It does this by bringing people and resources together, creating a community of students. These students identify web-based, evidence-based medicine (EBM) resources which are then categorised and commented upon, in turn directing users towards those of most interest and usefulness. The students also promote, discuss and criticise EBM by blogging about the resources they find. This could be anything from helpful teaching and training materials about medical statistics, to published systematic reviews, useful critical appraisal tools, economic evaluations or evidence databases.
We spoke with Holly Millward, the Community Lead for S4BE, for more information about the organisation.

Could you briefly tell us who you are and what you do?
Hi, I am Holly, I work at Cochrane UK as a Communications and Engagement Officer. As part of my role I support the running of Students 4 Best Evidence (S4BE). I also work for Cochrane as an Interim Brand and Communications Co-ordinator and I am Digital Communications Editor for Evidence-Based Mental Health.

What made you establish and continue to run S4BE?
Our director at Cochrane UK, Professor Martin Burton, set up S4BE with the aim of providing a middle step between students and Cochrane and other evidence-based organisations. The objective was to create a site that students can use to find links and resources that can provide them with information about evidence-based healthcare and how to put it into practice. S4BE was launched in May 2013 and we are really proud of how the community has grown and of the content that the S4BE contributors produce. The continued growth of S4BE is thanks to the amount of students globally who use the content every week.

What does 'evidence-based' mean?
The words "evidence-based" are used to describe lots of things in healthcare and beyond. Evidence-based medicine, evidence-based practice, evidence-based policy, and – in a different part of society – evidence-based social work and evidence-based education. The underlying principles are the same. The concept is about making sure that when decisions are made they are based on the most up-to-date, solid, reliable, scientific evidence. In the case of medicine or health care, these are decisions about the care of individual patients. Evidence-based medicine is about asking the right questions and using the best research evidence to answer those questions.

What are your current goals?
Our main goal at the moment is to increase the number of students contributing to the website. We also want student feedback on whether the website could be improved, to allow us to provide the most useful content students are looking for. Also in April, we have started the "What's the evidence 4 this?" campaign which aims to increase students' use and knowledge of evidence.

Could you tell us more about this campaign?
We have launched a year-long campaign to get health sciences students around the world to ask their educators, "what's the evidence 4 this?" (#whatstheevidence4this). The campaign started with a Thunderclap that was launched at Evidence Live (evidencelive.org) on the 13th of April.

What has S4BE already achieved? Do you have an example of something you are proud of?
S4BE contributors have done a fantastic job at communicating the best evidence and teaching others how to use this evidence. One blog that has done very well is Tim Hick's tutorial about odds ratios, p-values and confidence intervals (http://www.students4bestevidence.net/a-beginners-guide-to-interpreting-odds-ratios-confidence-intervals-and-p-values-the-nuts-and-bolts-20-minute-tutorial/). Since its publication in August 2013, this blog has provided 50% of all our page views and currently has 49 comments from students asking Tim for further help. This is fantastic and shows resources like this are in high demand.

Lately, there has been some criticism about research. Some people doubt the relevance of research; in other words, they are not sure whether every study is relevant enough. What are your views on this?
This is why systematic reviews are so important in pulling together all the studies on a particular topic to provide a more rounded answer. S4BE Contributor Danny Minkow has written a really useful blog explaining how systematic reviews work (http://www.students4bestevidence.net/systematic-review/).

How can students without any research experience contribute to S4BE?
S4BE is for any student, of any age, in any discipline. Whether you are completely new to the concept of evidence-based health care and want to blog about your initial thoughts on how you think evidence can be used, or if you have clinical experience of finding the best evidence and putting this into practice. We want to hear about it all if that is how we can help students to learn more. For those students who do not want to blog for us, but would still like to be involved, please comment and share our resources on social media and with your student communities. You can find us on Twitter, Facebook, Google Plus and YouTube. Now we have launched our year-long "What's the evidence 4 this?" campaign, we have lots of tasks that everyone can get involved with! So join the Students 4 Best Evidence Campaign and ask "What's the evidence for this?"

Are Dutch students able to contribute to S4BE?
Yes, all students no matter where you are based around the world can get involved! Please contact me if you would like to know more: general@students4bestevidence.net

DYSTONIA AND PSYCHIATRIC DISORDERS

A pathofysiological connection?

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ABSTRACT:

BACKGROUND: Combination of dystonia and psychiatric diseases is 91.4% in cervical dystonia. Common psychiatric disease differ per form of dystonia. We hypothesize a pathophysiological connection between psychiatric disorders and dystonia.
OBJECTIVE: This exploratory review focuses on the basal ganglia, as literature indicates that its dysfunction is related to either psychiatric disease and dystionia.
RESULTS: Although no specific mechanism was found, indications for a pathofysiological connection were identified. These indications include damage to parallel loops in the basal ganglia involved with the two diseases, an effect on serotonin via dopamine, a similar location in the brain and a primary link between psychiatric diseases and Parkinson's disease, which is also a motor disease. To identify a pathophysiological connection, additional research may be needed on this topic, for example combining other motor and non-motor diseases and try to relate their disease mechanism to dystonia.
CONCLUSION: The exact pathophysiological relationship between dystonia and psychiatric disorders remains unclear. Several theories have been proposed, but more research is needed to further elucidate the roles of different brain circuits.

WHAT IS KNOWN: There is a large correlation between dystonia and psychiatric disorders.

WHAT IS NEW: There might be a pathophysiological connection between psychiatric disorders and dystonia. This connection can possibly be found in the basal ganglia or the secretion of neurotransmitters. Additional research is needed.

KEYWORDS: Dystonia, psychiatry, basal ganglia, neurotransmitters

Introduction

Dystonia is a neurological movement disorder. Although basal ganglia dysfunction is commonly considered to be involved, precise pathophysiologic mechanisms are still unknown. Dystonia occurs in different forms, such as primary (i.e. no other feature than dystonia is involved) and secondary dystonia, for example due to a lesion of the basal ganglia or use of medication. An important characteristic of dystonia is that muscles are continuously strained, as will be further explained in the functional anatomy part.

There is a high co-incidence of dystonia and psychiatric diseases, being mainly depressions and anxiety disorders. In cervical dystonia for example, co-incidence of any psychiatric disease has been reported to be up to 91.4%, compared with 35% in the general population[1]. Furthermore, some dystonic patients are diagnosed with more than one psychiatric disorder. An epidemiological study[2] that included 28 dystonic patients showed that of dystonic patients with a major depressive disorder, 3 out of 7 also had to cope with a generalized anxiety disorder.

The fact that dystonia and psychiatric disease often go hand in hand, may suggest similarities in the underlying disease mechanisms, because of possible interactions between the disease mechanisms. If there are similarities in both disease mechanisms it is important to find these for different reasons. First, this would increase general knowledge of doctors, patients and society. For example, better understanding of dystonia-related psychiatric diseases will enhance acceptation of patients in society, instead of regarding psychiatric symptoms of dystonic patients as a result of their difficulty to cope with dystonia in daily life. Advanced patient education increases quality of life of patients, because they know what to expect. Second, the perspective on dystonia can be changed. Dystonia used to be seen as a purely physical disease. More and more, research shows involvement of non-motor brain elements. Dystonia can be recognized faster, and thus treatment can be started earlier, for a better outcome. Third, treatment of dystonia can possibly be broadened to treatment of psychiatric diseases. When the underlying mechanism of dystonia and psychiatric disease is linked, this could be an opportunity to implement treatment of psychiatric diseases in treatment of dystonia. In this explorative review, we analyze current knowledge about the pathophysiological link between dystonia and psychiatric diseases, to answer the following question: what is the role of basal ganglia and neurotransmitter (dys)function in the occurrence of psychiatric disorders as a co-morbidity in dystonic patients? To address this question, we searched for studies on comorbidity of psychiatric disorders and dystonia together with basal ganglia function and different neurotransmitters to summarize current knowledge on this topic.

Functional anatomy of the basal ganglia

In order to understand the mechanism of dystonia, we should be aware of the functional anatomy of the basal ganglia, along with the several existing circuits that connect it with the different parts of the brain. Knowledge on the basal ganglia can also give insight in the mechanism that plays a role in several related diseases with motor and non-motor symptoms, as will be discussed later in this review.

Throughout the past, the classification of dystonia shifted from being a psychogenic disorder towards an organic movement disorder[3]. Nowadays, dystonia is defined as "a syndrome of involuntary sustained or

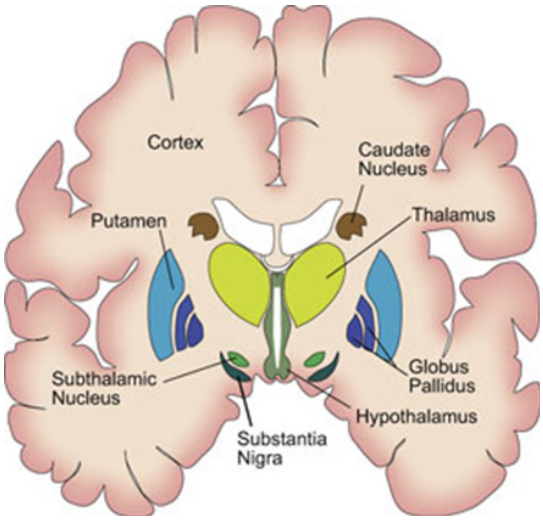

Figure 1 *Schematic representation of the major nuclei within the basal ganglia[16]. The different nuclei of the basal ganglia are spread through the brain, but mostly located in the inner part. The putamen, nucleus caudatus and substantia nigra form the outer border of the area where the basal ganglia are lain. The hypothalamus forms the center of this area. Basal ganglia are symmetrical.*

intermittent muscle contractions leading to twisting or repetitive movements or abnormal postures"[4]. The proposed core mechanisms of dystonia involve two principles: over-contraction of the muscles that normally are used for movements and over-flow contraction of muscles that could antagonize these primary movement muscles[5]. But what kind of mechanism is causing these abnormal muscle contractions? Research indicated that the basal ganglia play an important role in the onset of dystonia[6]. The basal ganglia are a group of nuclei that are located deep within the cerebral hemispheres, known for their role in movement control. The different nuclei of the basal ganglia are divided into functional subdivisions (Fig. 1). The caudate nucleus, nucleus accumbens and the putamen together form the corpus stratium. Two other structures of the basal ganglia are the external and internal globus pallidus and the subthalamic nucleus. Finally, the substantia nigra has a major influence on basal ganglia function, via its dopaminergic projections. Dopamine is the driving force of the basal ganglia and therefore needed for normal functioning of the basal ganglia[6]. Shortage or absence of dopamine is linked with movement abnormalities, including Parkinson's disease and some forms of dystonia[6]. The basal ganglia control movement initiation and suppression of unwanted motor activity by communicating with the cerebral cortex and the thalamus. The input for the basal ganglia comes from the cerebral cortex. The signals pass through different nuclei of the basal ganglia and communicate back to the cerebral cortex via the thalamus. This pathway is an example of the different functional loops that are present in the basal ganglia. In fact, there are many different loops present in the basal ganglia. These loops are grouped into three major functional categories that are functioning parallel to each other (Fig. 2). The first category is the motor loop. This loop communicates with the premotor cortex and is involved in the initiation and planning of movements. This motor circuit has a direct and indirect pathway in which it influences movement initiation and suppression. The direct pathway goes from the putamen to the internal globus pallidus and is responsible for the activation of movements. The indirect pathway is also going from the putamen towards the internal globus pallidus, but via the subthalamic nucleus and/or the globus pallidus externus. This pathway is responsible for the inhibition of movement. One could hypothesize that a defect or lesion in the motor circuit could result in an imbalance of the direct and indirect pathway. This imbalance could lead to over-contraction of primary muscles and over-flow contraction of secondary muscles, two principles by which dystonia is characterized. Control of movement is not the only function, because the basal ganglia are also involved in other processes, such as motivational, emotional, associative and cognitive processes[7]. The second and third loop, the associative and limbic loop, are responsible for these other processes. The associative loop is connected with the prefrontal cortex and may regulate the initiation and termination of cognitive processes such as planning, working, memory and attention. The limbic loop communicates with different parts of the limbic lobe of the cortex. This loop may be responsible for regulation of emotional and motivated behaviour and the reward system.

Figure 2 shows the motor and non-motor loops through the basal ganglia. In dystonic patients there are often psychogenic symptoms present besides the problems with movement. Because the loops of the basal ganglia are parallel to each other, we hypothesized that it may be possible that if one circuit is disturbed, the other two circuits also become affected, because of the connections between the three loops. This could explain that dystonia comes along with a lot of other psychological factors, for example depression or anxiety disorders.

Neurotransmitters

There are several hypotheses about the underlying mechanism of dystonia, although the exact causes remain elusive. One of the most prevailing hypotheses is that dysfunction of the basal ganglia is due to abnormal neurotransmitter function in the basal ganglia, with special

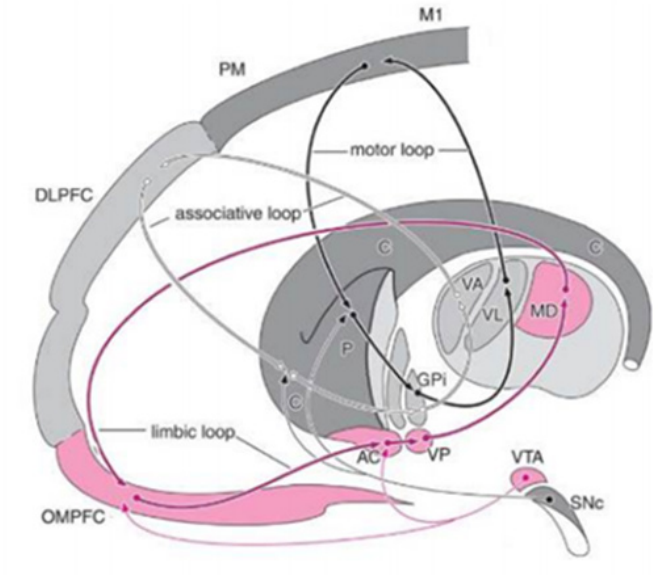

Figure 2 *Functional loops of the basal ganglia[17]. This figure shows different loops in which the basal ganglia are involved: the limbic loop, the associative loop and the motor loop. Abbreviations for the different parts that contribute to these loops are explained below.*

AC *Anterior commissure* ***C*** *Cortex* ***DLPFC*** *Dorsolateral prefrontal cortex* ***GPi*** *Globus pallidus interna* ***M1*** *Primary motor cortex* ***MD*** *Medial dorsal nucleus* ***OMPFC*** *Orbital and medial prefrontal cortex* ***P*** *Putamen* ***PM*** *Premotor area* ***SNc*** *Substantia nigra compacta* ***VA*** *Ventral anterior nucleus* ***VL*** *Ventral lateral nucleus* ***VP*** *Ventral pallidum* ***VTA*** *Ventral tegmental area*

attention to dopamine[11].
This section will be devoted to potential links between the mechanism underlying the motor facets of dystonia and the non-motor aspects of the disease. First of all, we will elucidate the several functions of dopamine on the motor aspects of dystonia. The second part of this first link will be dedicated to the interaction between dopamine and serotonin and the effect of this on the non-motor functions of the basal ganglia as serotonin is frequently involved in psychiatric diseases.[8] An effect of dopamine on serotonin could implicate an effect of dopamine on the motor system and an indirect effect of dopamine on the psychiatric situation of the patient. The section finishes with a hint to other neurotransmitter systems that could also play a role in dystonia.

Dopamine and the motor aspects of dystonia
There is substantial evidence that dopamine plays a crucial role in the development of the motor symptoms in dystonia. For example, DOPA-responsive dystonia (DRD), a disorder with a direct relationship to the lack of dopamine, responds very well to dopaminergic treatment. Several genes which are involved in the dopamine secreting and synthesis pathway have been linked with DRD, such as the gene encoding for guanine nucleotide binding protein, alpha activating activity polypeptide, olfactory type (GNAL)[9]. Of note, DRD might shed more light on the pathogenesis of other forms of dystonia than was previously thought [9]. The fact that several analogous genes for primary dystonia and DRD have been found could mean that there is a common causal pathway for primary dystonia and DRD[11]. The upcoming focus on dopamine as cause of motor symptoms in dystonia could be followed by a shift of attention to the effects of dopamine on the non-motor symptoms as well.

The effect of dopamine on serotonin
Another mechanism for dopamine to effect both motor and non-motor aspects of dystonia is through its effect on serotonin, which could be another link between motor and non-motor symptoms in dystonia patients. Serotonin is widely considered as an important neurotransmitter in the development of neuropsychiatric disorders. For example, the chronic use of levodopa, a drug used for treating Parkinson patients in order to replenish their dopamine level, reduces serotonin levels in the dorsal raphe nucleus[10]. Serotonin is widely known for its role in neuropsychiatric disorders[8]. Although levodopa is a precursor of dopamine, this does not mean that there is a direct negative relationship between the dopamine and serotonin levels because dopamine could also have an effect on other mechanisms that influence the level of serotonin. The abundance of dopamine might also influence other mechanisms such as neuroplasticity[11]. One must realize that dopamine is definitely not the one and only cause of either the motor or non-motor aspects of dystonia. The fact that there is a relationship between dopamine and serotonin, means that there may be an unidentified pathological mechanism in which dopamine and serotonin are involved. Moreover, the continuous findings of new pathways and mechanisms may imply that there are many other relationships to be uncovered and that there are, next to dopamine, several other neurotransmitters which play a role in dystonia and psychiatric disorders.

Other important neurotransmitters in the basal ganglia
Dopamine is obviously not the only neurotransmitter active in the basal ganglia. The three most important neurotransmitters of the basal ganglia are γ-Aminobutyric acid (GABA), glutamatic acid and dopamine[12]. GABA is the most important inhibitory neurotransmitter and glutamate is an excitatory neurotransmitter. Research has shown that disturbances in the glutamate level can lead to many different neuropsychiatric disorders such as schizophrenia and mood disorders[13]. Antagonizing the N-methyl-aspartate receptor (one of glutamate's main receptors) reduces the activity of inhibitory neurons, which then again results in the disinhibition of glutamate[15]. We could not identify any reports on the possible effects of GABA or glutamate on the motor functions in the human brain or in the basal ganglia. Possibly, future research could be done on these different neurotransmitters and their possible effect on either dopamine or directly on the motor loop in the basal ganglia.

Combined psychiatric and motor diseases

Possible mechanisms underlying the combination of psychiatric disease and dystonia have not been studied yet. A more studied example of psychiatric symptoms combined with motor disease is Parkinson's disease.

Depression related to Parkinson's disease
The depressive symptoms in Parkinson's disease are probably primary linked to the disease mechanism of Parkinson[14]. However, no linear correlation of depressive symptoms with duration or severity of Parkinson's disease was found. Assumably, depression in Parkinson's disease is caused by the degeneration of monoaminergic neurotransmitter systems. Another possibility is fronto-cortical dysfunction[15]. Nowadays, there is still a lack of knowledge of the pathofysiological mechanism of depression in Parkinson's disease. Although this is the case, it can be suggested that just like in Parkinson's disease, dystonia's non-motor symptoms are not a result of the dystonia but part of the disease's mechanism.

Discussion and conclusion

Dystonia is a complex disease and still very few is known about the causal mechanism behind the disease. In this review we chose to discuss the basal ganglia circuitry and its function, because these brain networks are seen as the key players in motor control and classically their dysfunction is linked to dystonia. We have discussed the three major loops that are present in the basal ganglia and how their parallel organization may lead to interaction; a potential cause of concomitant motor and non-motor symptoms in dystonia. We also hypothesized that dopamine and serotonin have a role in the occurrence of dystonia. Finally, other diseases with both motor and non-motor functions were discussed and compared to dystonia. We conclude that the mechanism of disease of dystonia probably not only leads to the obvious motor symptoms, but also has a result on non-motor functioning of the patient.

A limitation of this review is that we only discuss the basal ganglia, which does not show the complete picture of the disease. In fact, the view on dystonia is shifting towards a broader network disorder. The basal ganglia are not the only structures that are involved in movement initiation and control. For example, the cerebellum also plays a role in motor control and research showed that the cerebellum is connected to the basal ganglia. Furthermore, in our review we focused on the psychiatric co-morbidity in dystonia. We hypothesized that the basal ganglia are more involved in this co-morbidity than the cerebellum, as more scientific evidence can be found on the relationship between the basal ganglia and psychiatric disorders.

Another limitation is the large amount of different possible causes for the motor and non-motorsymptoms of dystonia. In our review, we focused on the several neurotransmitters which could possibly play a role in dystonia, because they are often associated with both motor and non-motor disorders. However, it could be possible that other factors or other neurotransmitters, besides the ones mentioned in our review, are involved in the onset of the motor and non-motor symptoms.

It should not be forgotten that the non-motor symptoms of dystonia possibly do not even share a similar disease mechanism as the motor symptoms. A possibility is that both symptoms have a common cause,

but not a common pathway. In this review, the emphasis is put on a common pathway for the motor and non-motor symptoms of dystonia. However, there are several other possibilities for the cause of the non-motor symptoms of dystonia. A reason for this hypothesis is that not all dystonia patients are affected by the non-motor aspects, which might indicate that either there are many different causes of dystonia, or the non-motor symptoms of dystonia are a psychological result of the motor aspects.

In conclusion, exact mechanisms about the onset of psychiatric disorders along with dystonia remain elusive. However, several potential links about these mechanisms were summarized in this review. Additional research on these links, for example on the basal ganglia interactions, need to be carried out in order to clarify the exact disease mechanisms. Clearly, more research is needed to further identify the links between dopamine and both the psychiatric and movement disorders in dystonia. Perhaps data on Parkinson's disease patients could also contribute here, since Parkinson's disease is caused by a shortage of absence of dopamine. Finally, it will be useful to further look at other combined motor and non-motor disease and try to relate their disease mechanism with dystonia.

References

1. Moraru E, Schnider P, Wimmer A, Wenzel T, Birner P, Griengl H, et al. Relation between depression and anxiety in dystonic patients: implications for clinical management. Depress Anxiety. 2002;16(3):100-3. Epub 2002/11/05.
2. Lauterbach EC, Freeman A, Vogel RL. Correlates of generalized anxiety and panic attacks in dystonia and Parkinson disease. Cogn Behav Neurol. 2003;16(4):225-33. Epub 2003/12/11.
3. Munts AG, Koehler PJ. How psychogenic is dystonia? Views from past to present. Brain. 2010;133(Pt 5):1552-64. Epub 2010/03/31.
4. Fahn S. Concept and classification of dystonia. Advances in neurology. 1988;50:1-8. Epub 1988/01/01.
5. Neychev VK, Gross RE, Lehericy S, Hess EJ, Jinnah HA. The functional neuroanatomy of dystonia. Neurobiol Dis. 2011;42(2):185-201. Epub 2011/02/10.
6. Wichmann T, Dostrovsky JO. Pathological basal ganglia activity in movement disorders. Neuroscience. 2011;198:232-44. Epub 2011/07/05.
7. Herrero MT, Barcia C, Navarro JM. Functional anatomy of thalamus and basal ganglia. Childs Nerv Syst. 2002;18(8):386-404. Epub 2002/08/23.
8. Nordquist N, Oreland L. Serotonin, genetic variability, behaviour, and psychiatric disorders--a review. Ups J Med Sci. 2010;115(1):2-10. Epub 2010/03/02.
9. Goodchild RE, Grundmann K, Pisani A. New genetic insights highlight 'old' ideas on motor dysfunction in dystonia. Trends Neurosci. 2013;36(12):717-25. Epub 2013/10/23.
10. Stansley BJ, Yamamoto BK. Chronic L-dopa decreases serotonin neurons in a subregion of the dorsal raphe nucleus. J Pharmacol Exp Ther. 2014;351(2):440-7. Epub 2014/09/13.
11. Fresnoza S, Stiksrud E, Klinker F, Liebetanz D, Paulus W, Kuo MF, et al. Dosage-dependent effect of dopamine D2 receptor activation on motor cortex plasticity in humans. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014;34(32):10701-9. Epub 2014/08/08.
12. Sian J YM, Riederer P, et al. Biochemical Anatomy of the Basal Ganglia and Associated Neural Systems. In: Siegel GJ, Agranoff BW, Albers RW, et al., editors. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Philadelphia: Lippincott-Raven; 1999. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27905/.
13. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 2012;37(1):4-15. Epub 2011/10/01.
14. Cummings JL. Depression and Parkinson's disease: a review. The American journal of psychiatry. 1992;149(4):443-54. Epub 1992/04/01.
15. Mayberg HS, Solomon DH. Depression in Parkinson's disease: a biochemical and organic viewpoint. Advances in neurology. 1995;65:49-60. Epub 1995/01/01.
16. Adam D. The Basal Ganglia. KIN450-Neurophysiology; Available from: http://kin450-neurophysiology.wikispaces.com/Basal+Ganglia+II.
17. Nieuwenhuys R, Voogd, J., Van Huijzen, C., van Huijzen, C., & Voogd, J. The Human Central Nervous System: Springer; 2008.

INNOVATION IN HEALTHCARE - INTERVIEW LUCIEN ENGELEN

By Anna van Boekel

A tablet on the wall, multiple iPhones on the desk, and a smart watch around his wrist; in front of us sits Lucien Engelen, the director of the Radboud REshape Center for Innovation. He introduced the Google-glass in the operating room, fights for patient empowerment and travels back and forth to Silicon Valley to look for new technologies which can be implemented in healthcare.

What is currently a 'hot topic' in healthcare?
'Do I really need to answer that in only an hour?'

I think there are four important developments in healthcare at the moment; my four D's: democratisation, decentralisation, digitalisation and the development of a free market. Simply, what we see is that patients are becoming more informed and they want to have their healthcare closer to their homes. Over the last few decades we have moved healthcare away from the people; instead of caring for individuals in their homes, people are brought to hospitals and institutions. New technologies will help to reverse this process by taking healthcare back to the people. These developments will influence the healthcare system globally and will lead to changes.'

Can you give us an example?
'One of the things that I think will happen is the development of a 'fourth line'. Care that was first provided by the general practitioner will go back to the patients themselves, and some of the care provided by the second line will go back to the general practitioner and so on. As a result the academic hospitals will get more and more complex patients, creating a fourth line.'

'Another development we are seeing is that people want to have more control over their own health and they are prepared to pay more for their healthcare if that means that they will receive a better service. New technologies will increase the possibilities in healthcare but it will also lead to situations whereby people with money are able to buy better healthcare than people without extensive financial resources, leading to ethical issues.'

Do you think that new technologies are able to decrease the gap between rich and poor?
'What normally happens is that a new technology is at first only available for the rich before everyone can afford it. This phenomenon is however needed for the development of new technologies. Some people try to stop it but that would be a critical blow to innovation. At the end of the day these products will be democratised, also from a financial point of view, and become more affordable for everyone. That is what happened with the internet and mobile phones, for example.'

Another concern many people have is that these developments will lead to loss of privacy and too much power for companies.
'The whole privacy mafia should be banished!

I think privacy and security are very important but at the moment there is too much restriction. We have proper legislation but we have to be careful that we do not let our developments be governed too much by the few cases that have gone wrong. Moreover, there are big companies involved in healthcare, such as Philips and Apple, which have something to lose when they make mistakes so they will be careful. Moreover, there is this tendency of governments and others to think there is such a thing as 100% secure and 100% private; that is utopia. There were crooks in the middle ages and there will be in the future, there will always be a hacker smarter than the smartest system'

What will be the consequence of all these developments in healthcare for the role of the doctor?
'The role of the doctor is changing. A doctor will become more a guide or a coach who will help patients to make their decisions.

Furthermore, data collection will become more and more important. We will be able to monitor people at home and collect huge amounts of data. Whereas researchers are now extremely happy with a study population of 1,000 or 10,000, we will be able to do research with 400,000 people. Consequently, the whole way we conduct our research is going to change. I do not condemn Evidence Based Medicine (EBM), do not misunderstand me, but it is always based on samples of a population and we never know if it works in real life. Now, for the first time in history, we will be present at the moment a person becomes ill. That has never happened before. It means that we are going to understand health and disease better which is of great value for public health and prevention.'

So you are able to collect data from healthy people and patients and this is going to change healthcare as well as biomedical research, but how are you going to collect this data? Do you imagine that in the future everyone will be wearing a 'health patch' or a 'smart watch' that will monitor them continuously?
'The health patch, a patch which can monitor patients 24/7, is one possibility. But take for example my watch.'

He shows us his watch which displays his heart rate and the number of steps he has walked last week.

'In the beginning I was continuously looking to see how many steps I had walked and I started to take the stairs instead of the elevator. Now, I am not even aware of it anymore but I still take the stairs and I would never have done it without the feedback from my watch. In the future the watch will also be able to keep track of other measures, such as my oxygen saturation (SpO2). Next time when I go to a doctor I can show him this data.

Figure 1 *Lucien Engelen, director of the Radboud REshape Center for Innovation*

With the collection of so much data people will get a lot of information about their own health. Do you not think this will lead to unnecessary worries and an increase in healthcare consumption?
'That will definitely happen. There will be an increase of what I coined 'digichondriacs'. We always tend to overshoot at first, but people know what they do and they will be careful. The technology is coming with algorithms to handle this, so we cannot ignore it. Patients are going to use it and, if you do not accept it and embrace this technology, they will go to a doctor that does.'

How fast do you think these developments will take place?
'I cannot do any predictions. It is a complicated process with so many different factors therefore it is hard to tell where we stand at the moment. But what is certain is that developments are going to happen. Also, it will happen sooner and faster than we expect, that is for sure.'

There are a lot of developments going on within healthcare and (future) doctors will have to deal with this. Is this also going to be implemented into the new curriculum?
'There will be a few subjects about new technologies and their possibilities. The interface of new technologies is becoming easier but the number of different technologies is increasing so what we have to learn is how to choose what is useful and what is not. Moreover we are also going to implement more about patient empowerment, involving patients in health care.'

Patient empowerment is something you fight for?
'Yes, involving patients in healthcare and the development of healthcare.'

Lucien Engelen introduced a chief listening officer in the Radboudumc, someone who does nothing else other than listening to the experiences and wishes of patients about the health policy. Another project he started is FaceTalk, a video conference-system that allows patients to communicate with their doctor through secure video software so they do not always have to come to the hospital. Moreover, he also involves patients in the development of healthcare and regularly asks patients for advice. His latest project is Hereismydata™, a new electronic platform for patients and doctors on which patients can upload data from health and fitness apps.

Do you also try to involve patients in research?
'Yes, we should try to involve patients in everything we do, including our research.'

How do you envision that?
'Patients can be involved in research at different levels. The first thing we can do is to ask patients before we start a research project if they think the research will be relevant or not. Moreover we also ask them what topics THEY want us to dive into. We try to involve patients from the start and ask along the way what they think of it, which really is an advantage. The next step, which we are launching right now, is that we are going to help patients to start their own research. In this project, called MedCrowdFund™, we will help them to write their own research proposals, to search for the necessary money and to hire a researcher to do the research. It is not going to happen 'en mass' but it will turn the research process upside down! A good example, that is now running, is the project 'Dance for Parkinson'. The director of the dance theatre of Tilburg has Parkinson's disease and wondered if dancing has a positive effect on his disease. So he started, with our help, a research project to determine the effects of dancing on Parkinson's disease. The number of comparable projects will increase with the rise of crowd funding.'

Could this also be a solution for the high pressure on research grants?
'The big funding bodies are not that eager because they prefer people donating to them, so they can then decide themselves how to use the money. However, people prefer to help people they know, I sometimes call it the 'Alpe d'Huez effect'. The cyclists that go up the Alpe d'Huez collect so much money because there is almost a direct relation between the cyclists and the people that donate instead of an anonymous collecting-bus. People like to know to whom they donate, it is a social phenomenon which I hope and expect we are going to utilise within the research world.'

In Nijmegen, with Radboud REshape, we are at the front of these developments. Do you think that we are ready for all these changes in healthcare; more technology and patient empowerment?
'No, the Netherlands and we at REshape, are not ready yet. We are currently at the point that about twenty percent of people are developing really fast but the vast majority of the population are at the beginning of the process. However, in the Netherlands we are one of the leaders in this field. In the United States for example, they are good in marketing and developing new technologies, but they do have issues implementing it into healthcare. That is what we, in the Netherlands, do well and I think we can be proud of it.'

'So we can be at the forefront of technology together with patients, but we must start these developments instead of just talking about it. The best way to change the future is to develop it yourself.'

DEVELOPMENT AND CHARACTERIZATION OF AN ANTIBACTERIAL ELECTROSPUN NANOFIBER WOUND DRESSING

Stefan JA Remmers MSc[1], Fang Yang, Jiankang Song

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ABSTRACT:

Supplementary tables can be found on our website

BACKGROUND: Wound dressings with non-antibiotic antibacterial properties are required to decrease the risk of wound infection, because the barrier-effect of standard dressings is insufficiently effective, and antibiotics lead to resistant bacterial strains
OBJECTIVE: To develop antibacterial electrospun membranes and determine their drug release kinetics and cytotoxicity.
METHODS: Chitosan-based nanofibrous membranes were electrospun with silver nanoparticles (AgNP's) from chitosan / polyethylene oxide / AgNO3 solutions, that were subsequently loaded with various amounts of chlorhexidine (CHX). Loading of CHX was expected to elicit a burst release while co-electrospinning AgNO3 together with chitosan was expected to result in AgNP's that provided a sustained release.
RESULTS AND DISCUSSION: Scanning electron microscopy showed chitosan-based membranes consisting of uniform and defect-free nanofibers. Transmission electron microscopy indicated the presence of AgNP's in the fibers with average diameters of 1.9 nm and 3.0 nm when adding 1% and 5% AgNO3, respectively. CHX was released within six hours while silver release was sustained, as determined by high performance liquid chromatography and inductively coupled plasma mass spectrometry respectively. Release of CHX and silver was in the same order of magnitude as many common minimal inhibitory concentrations (MIC's) in literature. Alamar Blue cytotoxicity assays showed that silver was not cytotoxic, whereas CHX was slightly cytotoxic at concentrations of 12.5 µg/ml and severely toxic above 25 µg/ml.
CONCLUSION: We were able to create chitosan-based membranes that elicited a strong CHX burst after loading and sustained release of silver over time. We created membranes that showed low or no cytotoxicity while the concentrations of released drugs were within range of various common MIC's. Future research should determine the synergetic antibacterial effect and the actual concentrations of antibacterials after application at the wound site.

WHAT IS KNOWN: Wounds are prone to infection. Current wound dressings are not effective enough to prevent wound infection. Antibacterial agents used in coatings or membranes could significantly reduce wound infections. None have succeeded in fabricating a wound dressing with three synergetic antibacterials.

WHAT IS NEW: We succeeded in developing a nanofiber wound dressing using three synergetic antibacterials. Different mechanisms of action of the antibacterials increase the effectiveness, yet limit the toxicity of the membranes.

KEY WORDS: Antibacterial Wound Dressing, Electrospinning, Chitosan, Silver, Chlorhexidine

List of Abbreviations: AA: Acetic acid, Ag: Silver, AgNP: silver nanoparticle, CHX: Chlorhexidine, DMSO: Dimethyl sulfoxide, FBS: Fetal bovine serum, HFF: human foreskin fibroblast, HPLC: High performance liquid chromatography, ICP-MS: Inductively coupled plasma mass spectrometry, MIC: minimal inhibitory concentration, PEO: Polyethylene oxide, PS: Pennicilin/streptomycin, SD: Standard deviation, SEM: Scanning electron microscopy, TEM: Transmission electron microscopy

Introduction

Common bandages and wound dressings serve as a barrier between wounds and the external environment, yet wounds remain prone to infection[1]. Therefore, a wound dressing is needed that possesses intrinsic antibacterial properties via incorporated antibacterial agents. The dressing should ideally elicit a burst release of the antibacterial agents to clear the wound of microorganisms, and a sustained release for keeping the wound free of microorganisms until the dressing is changed[2,3] typically at least once every 48 hours.

Electrospinning, an inexpensive, simple, yet effective technique for creating nanofibrous membranes has attracted attention as a method to create wound dressings[4,5]. Using electrospinning, antibacterial biodegradable non-woven nanofiber meshes can be fabricated from a viscous polymer solution[4,6].

Chitosan was electrospun in many previous studies[7-9] It is a biocompatible and biodegradable polymer that is abundant, cheap and has haemostatic[10] and antibacterial properties[11,12]. However, chitosan's antibacterial properties are limited, and the minimal inhibitory concentrations (MIC) against microorganisms commonly exceed 1000 µg/ml[13]. Electrospun chitosan membranes are not capable of sufficient antibacterial inhibition alone[14,15] but due to the biocompatible and biodegradable nature, they are an excellent drug-carrier material for targeted and timed drug delivery[14,15].

To improve the antibacterial properties of electrospun chitosan-based membranes, compounds such as antibiotics[16] or antiseptics can be used. A known problem with antibiotics is the development of resistant bacterial strains, and antibiotics should therefore not be used. Ideal antibacterial compounds should have antibacterial properties against a broad spectrum of bacteria, have MICs that are obtainable and have no or low toxicity.

Silver is a relatively inexpensive antiseptic that has been used for its wide range of antibacterial properties and low toxicity[17-21] for thousands of years. It is bactericidal at low concentrations (MIC range against most common microorganisms: 1.69 – 13.5 µg/ml)[22-25], and acts in synergy with chitosan[26]. Silver nanoparticles (AgNP's) are regarded as the most effective method to incorporate silver for antibacterial purposes due to their high surface-area-to-volume ratio[17-21]. To produce AgNP's, Ag+ ions are chemically reduced and stabilized inside the nanofibers by a polymer such as chitosan[15]. AgNP's have

successfully been used in electrospun antibacterial membranes[15, 27-29] and have shown good antibacterial effects within several hours[15]. However, gram positive bacteria are less susceptible to silver than gram negative bacteria[17,23]. Silver can be toxic at higher concentrations, but study design and AgNP characteristics differ widely and make comparing results of different toxicity studies virtually impossible[30].

Chlorhexidine (CHX) is a relatively inexpensive antiseptic mostly used for dental applications. It is a potent and fast acting bactericidal compound (MIC range against most common microorganisms: 0.25 – 30 µg/ml)[31-33]. In contrast to silver, it is most effective against gram positive bacteria[31,32]. CHX has also shown synergy with chitosan[34]. It has been used both as a component of fibers[35] or loaded onto fibers[36] to elicit an antibacterial response where it has shown a fast release pattern. In contrast to silver and chitosan however, CHX can be toxic at concentrations of 20 µg/ml or higher[37-39].

Individually, silver and CHX possess decent antibacterial properties. Combined with chitosan, the different mechanisms of action, different susceptibility of bacteria and synergy with chitosan may lead to an antibacterial membrane that is effective against a wide range of bacteria yet is not toxic, acts fast, and also maintains a sustained release of antibacterials. Surprisingly however, these three compounds have not been used together before for the fabrication of antibacterial electrospun chitosan-based nanofiber wound dressings.

In this study, chitosan-based electrospun nanofiber membranes were created by electrospinning chitosan with polyethylene oxide (PEO) [40] and acetic acid (AA). AgNP's were introduced into the fibers by adding $AgNO_3$ to the electrospinning solution. CHX was loaded onto the membrane after electrospinning. The aim of this study was to determine the effectiveness of the electrospun membranes as wound dressing by answering the following questions:

1. What are the release kinetics of electrospun silver and loaded CHX from the electrospun membrane over two days?
2. How is the cytotoxicity level of the membrane related to the incorporated amount of silver and CHX?
3. In which way does the simultaneous use of both silver and CHX affect the release of both compounds and the cytotoxicity level compared to using only one compound?

Materials and Methods

Materials

Chitosan (degree of deacetylation = 90%, molecular weight = 200-400 kDa, Heppe Medical Chitosan), Polyethylene oxide (Molecular weight = 900 kDa, Sigma-Aldrich®), chlorhexidine-digluconate (Sigma-Aldrich®) Acetic acid (99.9%, Boom BV, Netherlands), AgNO3 (Boom BV, Netherlands) and dimethyl sulfoxide (DMSO, Sigma-Aldrich®) were used as received. Donated Human Foreskin Fibroblasts were cultured in alpha Minimal Essential Medium (αMEM, Life Technologies, cat no. 22571) supplemented with 10% Fetal Bovine Serum (FBS, Sigma-Aldrich®) and 1% Penniciline/Streptomycin (PS, Sigma-Aldrich®).

Solution preparation for electrospinning

Four solutions were prepared for electrospinning. First, 2.25% (weight/volume, w/v) chitosan and 0.75% (w/v) PEO were added to a liquid phase of 25 vol% AA and 75 vol% ultrapure MilliQ grade water (MilliQ). To each solution, one of the following four $AgNO_3$ concentrations was added to create the final electrospinning solution. To create an 'empty', 'low silver', 'medium silver' and 'high silver' membrane, no $AgNO_3$, $AgNO_3$ concentrations of 0.1 wt%, 1.0 wt% and 5.0 wt% of the total polymer weight of chitosan and PEO were added to the solutions respectively. Solutions were stirred over night to ensure complete dissolution of the polymers and silver salts.

Electrospinning

Solutions were loaded in a 10 ml syringe (DB Plastipak). A silicon tube was connected to the syringe and the spinneret of a custom-made electrospinning apparatus, and the syringe was placed into a syringe pump (KD scientific). Aluminum foil was placed over a conducting drum (length: 16 cm, circumference: 25 cm) to facilitate fiber collection, and the distance between the spinneret and collector was set to 15 cm. Solutions were spun at a controlled flow rate of 4 ml/h at 27 kV on a drum rotating at 35 rpm while making a cyclic horizontal movement. The membranes were left to dry by air for at least one night, and were then stored at -20°C until further use. Samples were created by punching out discs with a surface area of 1 cm^2 from the membranes. The samples were weighed and samples with a similar weight were chosen for the following experiments. Average weights of the samples allocated to each group are displayed in Tables 1 and 2. In order to improve the mechanical properties of the membranes and reduce water solubility, fibers were cross-linked by treatment with glutaraldehyde in a vacuum vapor chamber for 40 hours[41]. This resulted in four groups with different membranes: a control group without silver, and three groups with different silver content. The silver content of all experimental groups containing silver and the control group is displayed in Table 1.

Loading of Chlorhexidine

From the four types of membrane, six more groups were created by loading CHX onto electrospun membranes. Chlorhexidine-digluconate stock solution was diluted to three concentrations: 4 mg/ml, 12 mg/ml and 20 mg/ml and samples were placed in 1.5 ml Eppendorf tubes (release kinetics experiment) or 24-well plates (cell toxicity experiment). Three groups containing a low, medium and high amount of CHX were created by loading 5 µl of the 4 mg/ml, 12 mg/ml or 20 mg/ml CHX solution onto control membranes respectively. Three more groups containing a fixed CHX content but different silver concentration were created by loading 5 µl of 12 mg/ml CHX onto 'low silver', 'medium silver' and 'high silver' membranes. This resulted in a total of ten sample groups: one control group without silver, three groups with different silver content, three groups with different CHX content, and three interference groups with different silver content and a fixed CHX content. All membranes were stored at 4°C over night to ensure proper absorption. The next day, the samples were freeze-dried for at least 24 hours. The CHX content of all experimental groups containing CHX and the control group is displayed in Table 2.

Membrane characterization

Fiber morphology was investigated using a scanning electron microscope (SEM, JEOL JSM-6340F). Nanofibers were deposited onto aluminum foil, cut and mounted on aluminum stubs using double-sided carbon tape and sputter-coated with gold for 60 seconds. Fiber diameter was determined by selecting 20 fibers randomly per image from five images, and measuring the average diameter using Image-J (NIH) software. Transmission electron microscopy (TEM, JEOL JEM-1010) was used to determine the presence and morphology of silver nanoparticles. Samples were collected by briefly placing copper sample support grids on the collector of the electrospinning device. Image-J software was used to determine the average diameter of the nanoparticles by measuring the diameter of 50 nanoparticles per sample.

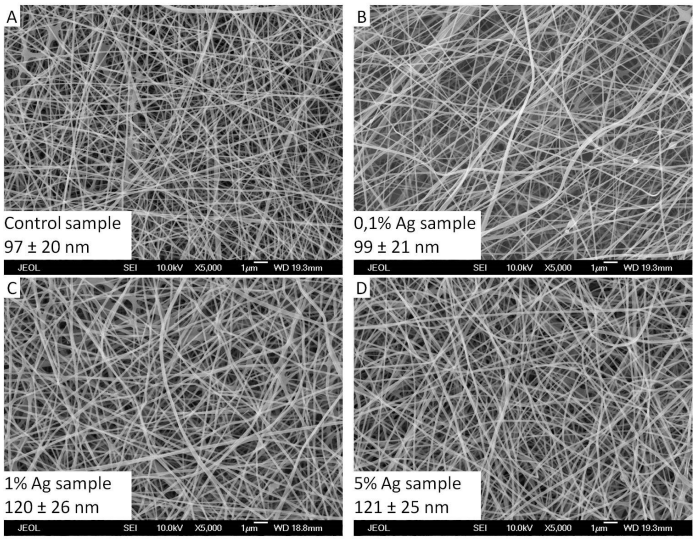

Figure 1 *Fiber morphology. SEM images of Electrospun chitosan / PEO nanofiber membranes illustrate the effect of AgNO3 addition on fiber diameter and morphology. (A) Control sample. (B) Sample with 0.1% AgNO3. (C) Sample with 1.0% AgNO3. (D) Sample with 5.0% AgNO3. All conditions produce uniform and defect-free fibers. With higher concentrations of AgNO3, fiber diameter tends to increase (but not significantly) and fibers seem to become more curly.*

Release kinetics test

Silver and CHX release from the membranes was measured at t = 6 hours, 1 day and 2 days (n = 5). Each sample was placed in an eppendorf tube (one sample per tube) and 1 ml of MilliQ was added to each tube. At each time point, 0.9 ml of liquid was removed and stored at 4°C for analysis and immediately replaced with 0.9 ml fresh MilliQ. The silver content was determined using inductively coupled plasma mass spectroscopy (ICP-MS, Thermo Electron Corporation, X series) using a sample matrix of 1.0% HNO_3. High Performance Liquid Chromatography (HPLC) was used to detect CHX release[42]. A mobile phase consisting of a 40% acetonitrile solution in MilliQ with 0.1% trifluoroacetic acid and 0.1% triethylamine was loaded into the HPLC device (Hitachi L-2130 pump, Hitachi L-2400 UV detector, Hitachi L-2200 auto sampler, Lichorspher RP-18 endcapped column). The flow rate was set to 1 ml/min, the injection value to 30 µl, and absorption was measured at λ = 260 nm. CHX retention time was approximately 3.1 minutes.

Cytotoxicity

Human Foreskin Fibroblasts (HFF) were thawed at passage 21 and cultured until passage 24 in culture medium aMEM supplemented with 10% FBS and 1% PS. Each of the 10 membrane sample groups contained three samples. First, an antibacterial medium was created by immersing each sample in 2.5 ml culture medium in 24-well plates for 24 hours (one sample per well). At the same time HFF cells in normal culture medium were placed into other 24-well plates (50000 cells/well) and were left to attach to the plate for 24 hours. After 24 hours, the medium was replaced with 1 ml of the new conditioned antibacterial medium (in duplo). Fresh medium and 5% DMSO were used as positive and negative control respectively. In addition, dilution series of CHX starting at 100 µg/ml and silver starting at 50 µg/ml was made in fresh medium and was tested for cytotoxicity simultaneously. The cells were incubated in the antibacterial medium or dilution series for 20 hours. Cytotoxicity was assessed with an Alamar Blue assay. The antibacterial medium was replaced by 1 ml culture medium containing 10% Alamar Blue dye. The cells were incubated for 4 hours wrapped in aluminum foil in an incubator. Clean medium with 10% Alamar Blue was used as control. After 4 hours, two 200 µl aliquots of metabolized medium from each well were placed in a transparent flat-bottom 96-well plate and fluorescence was measured at λ = 530/590 in a Bio-Tek® FL600 microplate fluorescence reader.

Statistical analysis

Sample groups were compared using a one-way ANOVA with Bonferroni correction for multiple testing. Means and standard deviation (SD) are presented in the following way: mean ± SD.

Results

Membrane characterization

Figure 1 displays SEM images of the electrospun chitosan-based membranes with different silver content. All membranes consisted of uniform and defect-free nanofibers. The diameters of the fibers tended to increase as the silver concentration increased. The diameters of the control (no silver) and 0.1% silver fibers differed significantly from the 1% silver and 5% ($p < 0.001$) silver fibers. While the fibers in the control sample seemed to be straight, the fibers tended to become curlier as more silver was added to the solution.

The presence of silver nanoparticles was investigated using TEM (Figure 2). No AgNP's were detected in the control fibers or the fibers containing 0.1% of AgNO3 (Figure 2A and B). For the samples containing 1.0 and 5.0% AgNO3 (Figure 2C and D), AgNP's had an average diameter of 1.85 ± 0.50 nm and 2.97 ± 0.55 nm, respectively. Higher concentration of $AgNO_3$ resulted in higher amount of AgNP's presented in the fibers.

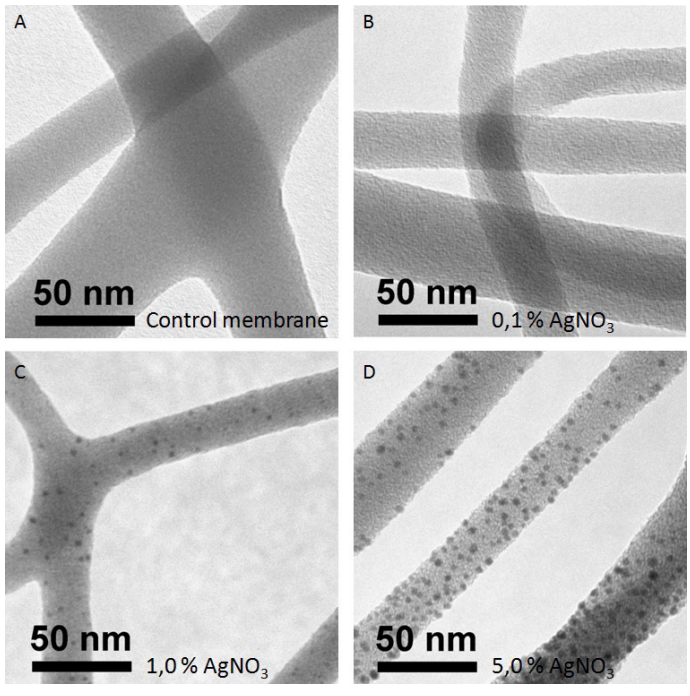

Figure 2 *Silver nanoparticle detection. TEM images of electrospun chitosan-based nanofibers with 0% (A), 0.1% (B), 1.0% (C) and 5.0% AgNO3 (D). No AgNP's were detected in the control fibers or the fibers containing 0.1% AgNO3. AgNP's in fibers with 1 and 5% AgNO3 had an average diameter of 1.85 ± 0.50 and 2.97 ± 0.55 respectively. Fibers with 5% AgNO3 contained more AgNP's than fibers with 1% AgNO3.*

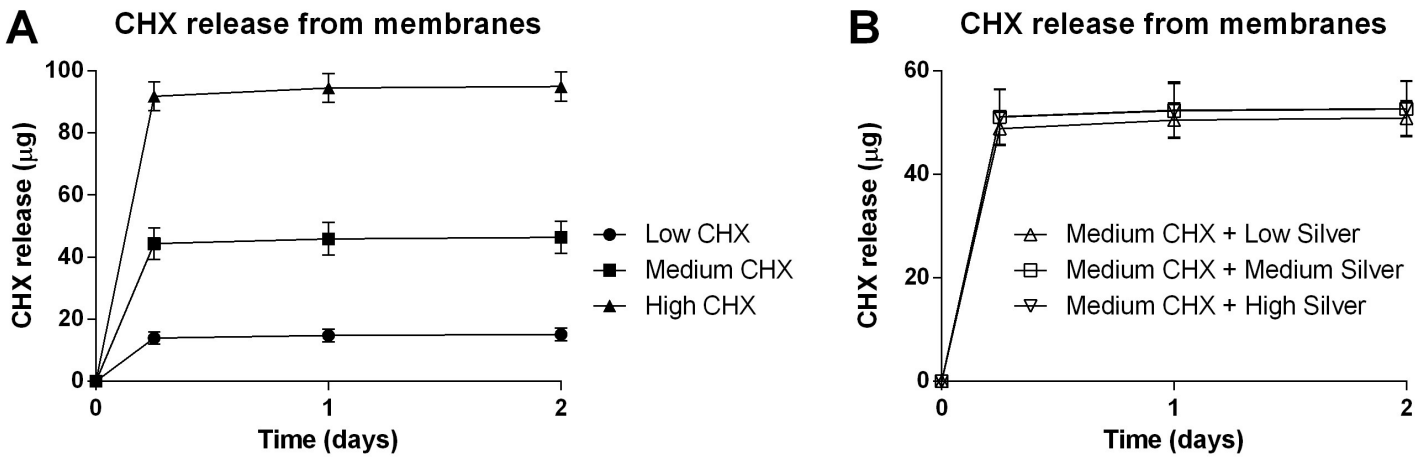

Figure 3 *Chlorhexidine release. These figures display the CHX release of the three membranes with different concentrations of CHX (A) and the CHX release of three membranes with identical CHX concentration but different silver content (B). All conditions show a burst release of CHX within the first 6 hours. Membranes with different CHX concentrations showed a burst at the same time, but reached a different cumulative release. The presence of silver in the membranes did not affect the release of CHX.*

Release kinetics of antibacterial compounds

CHX and silver release from the membranes in water was measured at different time points over a period of two days using HPLC (for CHX) and ICP-MS (for Ag). The results of the CHX release tests are presented in Figure 3. All six membrane groups that contained CHX gave a burst release of CHX within the first 6 hours, the height of which was dependent on the amount of CHX added to the membranes. The low CHX, med CHX and high CHX membranes released respectively 75%, 77% and 95% of the total CHX that was loaded onto the membranes. Figure 3B shows that the silver content of membranes did not influence the CHX release characteristics, although the percentage released for the three membranes with both silver and CHX is slightly higher than that of the membrane with the same CHX content but without silver (77% versus 87% on average). However, this difference was not significant ($p = 0.17$, $p = 0.61$ and $p = 0.60$ when comparing the membranes with only CHX to the ones with CHX and low, medium and high silver content respectively). The CHX content, 6 hour, 1 day and 2 day CHX release from the membranes are displayed in Table 2.

The silver release is presented in Figure 4 as detected by ICP-MS. The results were transformed to represent $AgNO_3$ release as opposed to silver ion release to make them comparable to the quantities incorporated into the membranes. This figure displays a strong sustained release that gradually weakens during the next two weeks. The membranes with different concentrations of silver showed corresponding release profiles; the membranes with the lowest concentration released the least amount of silver and the membranes with the highest concentration released the highest amount of silver. No significant difference in silver release was found between the membranes without CHX and corresponding membranes with CHX ($P < 0.05$). Cumulative silver release averaged over all membranes over 2 days was 0.91 µg, 12.1 µg and 40.6 µg for the membranes with low, medium and high silver content respectively. The silver content, 6 hour, 1 day, and 2 day cumulative release of silver from the membranes are displayed in Table 1. A logarithmic regression curve could be fitted to the individual groups ($R2 > 0.97$) using Microsoft Excel software, but no formula could be created that accurately predicted the sample data.

Cytotoxicity

Cytotoxicity of the membranes was measured by incubating them in fresh culture medium for 24 hours and culturing HFF cells in the conditioned medium. An Alamar Blue assay was used to quantify metabolic activity to determine the relative number of live cells. The results of the Alamar Blue assay are displayed in Figure 5. These results show no significant difference between the positive control and the metabolic activity of cells cultured in medium derived from the control membrane, 'low Ag', 'med Ag', 'high Ag' and 'low CHX' samples, indicating that these membranes were not cytotoxic. No significant difference was found between the negative control and the 'high CHX' group, indicating that these membranes are as cytotoxic as DMSO, the control sample. Samples cultured in medium derived from 'med CHX' containing membranes were significantly different from both the positive and negative control ($p < 0.001$) and hover

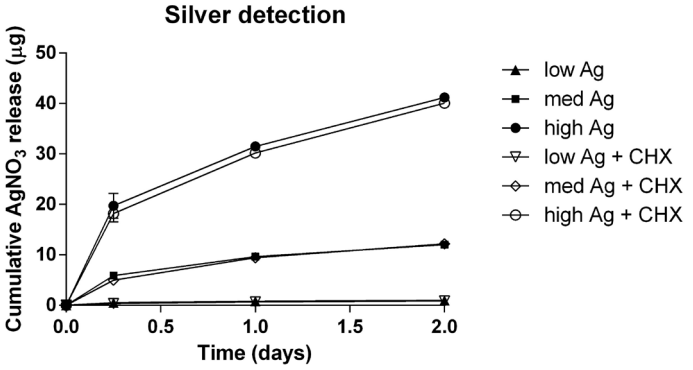

Figure 4 *Silver detection over time from membranes incubated in water for a period of 2 days. The figure clearly shows a strong burst release over the first six hours, and a steady but weaker gradual release over the next days. The presence of CHX on the membranes did not influence the release profile of Ag. The three used silver concentrations show three distinct release profiles, where the lowest concentration releases the least amount of silver and the highest concentration releases the highest amount of silver. Average cumulative silver release over 1 day was 0.76 µg, 9.53 µg and 30.84 µg and over 2 days was 0.91 µg, 12.1 µg and 40.6 µg for the membranes with low, medium and high silver content respectively.*

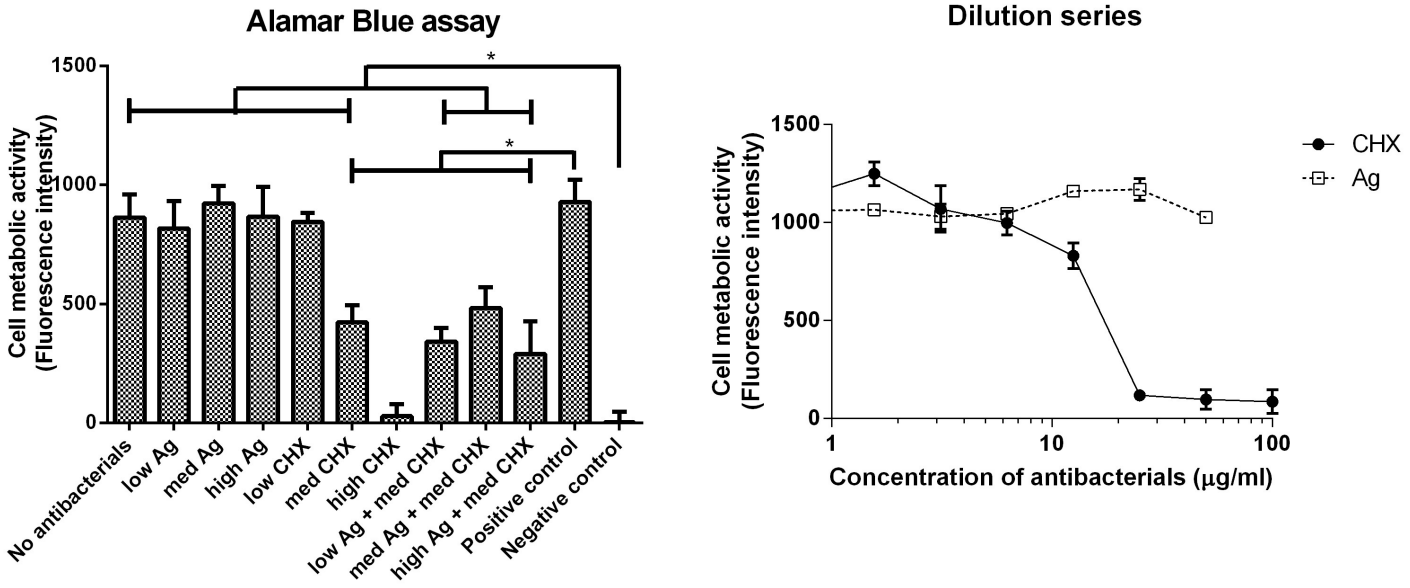

Figure 5 *Alamar Blue cytotoxicity assay (left). This figure displays the cell metabolic activity of cells after 24 hours of culture in antibacterial medium. Significance (P < 0.05) between a controlgroup and sample group is displayed as an asterisk above the figure. We found no significant difference between the positive control and the cell metabolic activity of cells cultured in medium derived from the control membrane, 'low Ag', 'med Ag', 'high Ag' and 'low CHX' samples. The negative control and the 'high CHX' group did not show a significant difference. Samples cultured in medium derived from 'med CHX' containing membranes showed a reduced number of cells and were significantly different from both the positive and negative control. Although they hover around the same values, some were shown to be significantly different from each other as well (right). The dilution series displays the cytotoxicity of a series of increasing silver or CHX concentrations. The silver dilution samples were not significantly different from the control sample. The first significant difference in the CHX dilution series with the positive control was found at 12.50 µg/ml. From 25 µg/ml and higher, no significant difference was found between the measurements and the negative control.*

around the same value, indicating they are cytotoxic, but there are still live cells present. The silver dilution series showed us that silver is not cytotoxic at the concentrations used in this study. When used against HFF cells, CHX has a mild cytotoxic effect at a concentration of 12.50 µg/ml. From 25 µg/ml and up, no significant difference was found between the measurements and the negative control, indicating the death of all cells.

Discussion

The aim of this study was to develop and characterize antibacterial membranes utilizing chitosan, silver and CHX that could emit a quick burst of antibacterials and a sustained release of antibacterials over time. We were able to create membranes that were not toxic to human fibroblasts, and showed a strong burst release of CHX followed by a sustained release of silver over time that achieved concentrations around and above most common MIC's found in literature.

SEM images showed that uniform and defect-free nanofibers were created. The membranes with silver contents of 1% or higher contained silver nanoparticles whose size and number increased with increasing $AgNO_3$ concentrations. No AgNP's were detected in fibers with 0.1% $AgNO_3$, either because the detection method is not accurate enough to detect nanoparticles of such a small size, or because the amount of $AgNO_3$ was not sufficient to induce nanoparticle formation.

Release kinetics showed a burst release of CHX within six hours and a sustained but weakening release of silver over time. CHX was released quickly, because it was loaded onto the fibers and was absorbed without creating molecular bonds between the chitosan or PEO molecules. Submersion in liquid caused the CHX to be released from the fibers quickly.

Silver was incorporated into the fibers through electrospinning, and could be released in one of two ways. Firstly, silver ions (Ag+) that are not or no longer part of nanoparticles can diffuse from the nanofibers because silver ions are much smaller than chitosan or PEO molecules. Secondly, degradation of the chitosan/PEO fibers can release silver ions and particles trapped inside. The strong initial release of silver was likely caused by diffusion of silver ions on or near the surface of the fibers, showing a Fickian diffusion profile[43]. The sustained release was likely caused by a combination of Fickian diffusion and slow degradation. However, the role of degradation is expected to be minimal, as the membranes showed no visible degradation in structure after incubation for four weeks (data not shown). In addition, investigation of the interference groups with both silver and CHX showed that the presence of either compound in the membranes did not influence the release characteristics of the other compound.

The cytotoxicity test showed that both the silver dilution series and the conditioned medium derived from silver containing membranes were not cytotoxic at any of the tested concentrations. CHX became mildly toxic at concentrations of 12.5 µg/ml, and severely toxic at 25 µg/ml, as was detected with the CHX dilution series.

Combining the results from the release kinetics and cytotoxicity tests, the antibacterial medium used for the cytotoxicity tests contained an estimated concentration of CHX of approximately 6.0, 18.6, and 38 µg/ml for the low, medium and high CHX membranes respectively. Of these media, the 18.6 µg/ml (med CHX) medium was shown to be moderately toxic, while the 38 µg/ml (high CHX) was severely toxic. These results indicate that the cytotoxicity of CHX starts around

12.5 µg/ml, is moderately toxic around 18.6 µg/ml and severely toxic above 25 µg/ml. These results are in agreement with literature which indicated that CHX is cytotoxic at concentrations of 20 µg/ml[37-39] and higher. Our results showed cytotoxicity at a lower concentration which can be explained by the use of different cell types (HFFs versus chondrocytes or odontoblast-like cells in literature) and longer exposure times (24 hours versus up to 2 hours in literature)[37-39]. The toxicity of the interference groups containing both silver and CHX was compared with the groups containing only CHX. Toxicity of the interference group did not differ significantly from the corresponding CHX groups, indicating that the presence of silver does not affect the toxicity of CHX, or vice versa.

To investigate the antibacterial effectiveness of the membranes a hypothetical scenario was created: the membranes were applied to a wound for 24 hours, and antibacterials were released into an exudate volume of 1 ml. Comparing the 1 day release of CHX (Table 1) with the MIC's that were found in literature, both the low CHX and medium CHX membranes would fall within the range of common MIC's, and the high CHX membrane would exceed this range. The medium and high CHX membranes however are cytotoxic in this scenario. The low CHX membrane would not be toxic and would fall within the range of most common MIC's, indicating that it is possible to fabricate membranes loaded with CHX that have no cytotoxicity but are lethal to variety of microorganisms.

The incorporated silver was shown to be nontoxic to HFF cells at all tested concentrations. The MIC's of silver for most common microorganisms are in a range between 1.69 and 13.5 µg/ml. In contrast to CHX, which was released as a burst within six hours, silver was released gradually. Using the hypothetical scenario in which the membrane is placed on a wound for 1 day and the antibacterials are spread over 1 ml, the low Ag membrane would not reach the lower limit of MICs found in literature. The medium silver membranes would be well within the range of the MICs found in literature, while the high silver membranes would exceed the aforementioned range.

Translating the results to be accurate in in vivo circumstances will be a challenge. In the experiments, membranes were incubated in water to examine drug release, but the in vitro release in water does not necessarily correspond to the in vivo release in wound exudate[3]. However, silver ion detection required the use of MilliQ because the chloride salts in PBS, culture medium or simulated wound fluid would bind to the silver ions and precipitate[2]. This would lead to an inhomogeneous silver distribution in the tubes that would lead to false ICP-MS results if the 'supernatant' is analyzed.

In addition, toxicity of membranes was calculated by submersing the membrane in culture medium (2.5 ml / cm^2) to create the conditioned medium. The volume to surface area ratio over which released antibacterials are spread when the membrane would be used in vivo is not fixed. There are no standardized values to use, and this ratio is dependent on many variables such as wound type, size, location, tissue vascularization and exudation. To determine this ratio for a specific would type, in vivo tests should be conducted.

In addition to CHX and silver, chitosan has antibacterial properties as well, but chitosan release kinetics were not investigated in the present study. The antibacterial activity of all three compounds has been tested multiple times in literature, but never together. Concentrations of antibacterials were achieved that are, according to MIC's found in literature, capable of inhibiting the growth of a variety of microorganisms. However, the MIC's found in literature differ widely among authors, especially for AgNP's, as the effect of AgNP's is dependent on the preparation method, size, shape and environment[17]. We hypothesize that these antibacterials can complement each other and have a synergic effect against a variety of microorganisms when used simultaneously, because all three antibacterials act through different mechanisms[26,33]. However, the release profiles of the tested compounds are considerably different, limiting the period in which the synergetic effect takes place to the first few hours. To investigate the net effect of the synergetic use of these compounds, additional in vitro tests are required that focus on testing the antibacterial properties of membranes containing various combinations and concentrations of the antibacterial compounds.

Conclusion

In this study, nanofibrous membranes were created from three synergetic antibacterial compounds. The membranes elicited a strong burst release as well as a sustained gradual release of antibacterials, effective against a wide range of bacteria at concentrations that are not toxic to human fibroblasts. However, because obtained results were compared with MIC's found in literature, additional in vitro tests need to investigate the antibacterial effectiveness of all three antibacterial compounds and their synergetic effect, and in vivo studies are required to investigate the actual released concentrations of antibacterials that the membranes can reach after application to a wound. Further investigation and development of these membranes could lead to affordable antibacterial wound dressings that are more effective than regular dressings in preventing wound infections.

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References

1. Dumville JC, Gray TA, Walter CJ, Sharp CA, Page T. Dressings for the prevention of surgical site infection. The Cochrane database of systematic reviews 2014; 9: CD003091.
2. Canada TA, Wiencek KM, Cowan ME, Lindsay BJ. Challenging Silver – Influence of Extraction Medium on the Release of Silver from Commercial Silver Dressings. In: LLC MHP, editor. Spartanburg: Milliken Healthcare Products LLC; 2007.
3. Jakobsen C. Relation of silver release and antimicrobial effect in-vitro of silver containing wound dressings: Linköpings universitet; 2010.
4. Bhardwaj N, Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnology advances 2010; 28(3): 325-47.
5. Li CW, Fu RQ, Yu CP, et al. Silver nanoparticle/chitosan oligosaccharide/poly(vinyl alcohol) nanofibers as wound dressings: a preclinical study. Int J Nanomed 2013; 8: 4131-45.
6. Li D, Xia YN. Electrospinning of nanofibers: Reinventing the wheel? Adv Mater 2004; 16(14): 1151-70.
7. Klossner RR, Queen HA, Coughlin AJ, Krause WE. Correlation of chitosan's rheological properties and its ability to electrospin. Bio-

macromolecules 2008; 9(10): 2947-53.
8. Desai K, Kit K, Li J, Zivanovic S. Morphological and surface properties of electrospun chitosan nanofibers. Biomacromolecules 2008; 9(3): 1000-6.
9. Zhang YZ, Su B, Ramakrishna S, Lim CT. Chitosan nanofibers from an easily electrospinnable UHMWPEO-doped chitosan solution system. Biomacromolecules 2008; 9(1): 136-41.
10. Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. The Journal of trauma 2003; 54(1): 177-82.
11. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 2003; 4(6): 1457-65.
12. Koburger T, Hubner NO, Braun M, Siebert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. The Journal of antimicrobial chemotherapy 2010; 65(8): 1712-9.
13. Goy RC, Britto D, Assis OBG. A Review of the Antimicrobial Activity of Chitosan. Polímeros: Ciência e Tecnologia 2009; 19(3): 7.
14. Dilamian M, Montazer M, Masoumi J. Antimicrobial electrospun membranes of chitosan/poly(ethylene oxide) incorporating poly(hexamethylene biguanide) hydrochloride. Carbohyd Polym 2013; 94(1): 364-71.
15. An J, Zhang H, Zhang JT, Zhao YH, Yuan XY. Preparation and antibacterial activity of electrospun chitosan/poly(ethylene oxide) membranes containing silver nanoparticles. Colloid Polym Sci 2009; 287(12): 1425-34.
16. Genuit T, Bochicchio G, Napolitano LM, McCarter RJ, Roghman MC. Prophylactic chlorhexidine oral rinse decreases ventilator-associated pneumonia in surgical ICU patients. Surgical infections 2001; 2(1): 5-18.
17. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of applied microbiology 2012; 112(5): 841-52.
18. Kumar R, Munstedt H. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials 2005; 26(14): 2081-8.
19. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science 2004; 275(1): 177-82.
20. Maaskant JM, De Boer JP, Dalesio O, Holtkamp MJ, Lucas C. The effectiveness of chlorhexidine-silver sulfadiazine impregnated central venous catheters in patients receiving high-dose chemotherapy followed by peripheral stem cell transplantation. European journal of cancer care 2009; 18(5): 477-82.
21. Damm C, Munstedt H, Rosch A. The antimicrobial efficacy of polyamide 6/silver-nano- and microcomposites. Mater Chem Phys 2008; 108(1): 61-6.
22. Panacek A, Kvitek L, Prucek R, et al. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. J Phys Chem B 2006; 110(33): 16248-53.
23. Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM. Silver Nanoparticles in Therapeutics: Development of an Antimicrobial Gel Formulation for Topical Use. Mol Pharmaceut 2009; 6(5): 1388-401.
24. Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta 2005; 51(5): 956-60.
25. Lkhagvajav N, Yasa I, Celik E, Koizhaiganova M, Sari O. Antimicrobial Activity of Colloidal Silver Nanoparticles Prepared by Sol-Gel Method. Dig J Nanomater Bios 2011; 6(1): 149-54.
26. Norowski PA, Courtney HS, Babu J, Haggard WO, Bumgardner JD. Chitosan coatings deliver antimicrobials from titanium implants: a preliminary study. Implant dentistry 2011; 20(1): 56-67.
27. Son WK, Youk JH, Lee TS, Park WH. Preparation of antimicrobial ultrafine cellulose acetate fibers with silver nanoparticles. Macromol Rapid Comm 2004; 25(18): 1632-7.
28. Penchev H, Paneva D, Manolova N, Rashkov I. Electrospun hybrid nanofibers based on chitosan or N-carboxyethylchitosan and silver nanoparticles. Macromolecular bioscience 2009; 9(9): 884-94.
29. Jin WJ, Lee HK, Jeong EH, Park WH, Youk JH. Preparation of polymer nanofibers containing silver nanoparticles by using poly (N-vinylpyrrolidone). Macromol Rapid Comm 2005; 26(24): 1903-7.
30. Beer C, Foldbjerg R, Hayashi Y, Sutherland DS, Autrup H. Toxicity of silver nanoparticles - nanoparticle or silver ion? Toxicol Lett 2012; 208(3): 286-92.
31. Block C, Furman M. Association between intensity of chlorhexidine use and micro-organisms of reduced susceptibility in a hospital environment. J Hosp Infect 2002; 51(3): 201-6.
32. Buxbaum A, Kratzer C, Graninger W, Georgopoulos A. Antimicrobial and toxicological profile of the new biocide Akacid plus((R)). J Antimicrob Chemoth 2006; 58(1): 193-7.
33. Rolla G, Melsen B. On the mechanism of the plaque inhibition by chlorhexidine. Journal of dental research 1975; 54 Spec No B: B57-62.
34. Decker EM, von Ohle C, Weiger R, Wiech I, Brecx M. A synergistic chlorhexidine/chitosan combination for improved antiplaque strategies. Journal of periodontal research 2005; 40(5): 373-7.
35. Lin YN, Chang KM, Jeng SC, Lin PY, Hsu RQ. Study of release speeds and bacteria inhibiting capabilities of drug delivery membranes fabricated via electrospinning by observing bacteria growth curves. Journal of materials science Materials in medicine 2011; 22(3): 571-7.
36. Chen L, Bromberg L, Hatton TA, Rutledge GC. Electrospun cellulose acetate fibers containing chlorhexidine as a bactericide. Polymer 2008; 49(5): 1266-75.
37. Campbell J, Filardo G, Bruce B, et al. Salvage of contaminated osteochondral allografts: the effects of chlorhexidine on human articular chondrocyte viability. The American journal of sports medicine 2014; 42(4): 973-8.
38. de Souza LB, de Aquino SG, de Souza PP, Hebling J, Costa CA. Cytotoxic effects of different concentrations of chlorhexidine. American journal of dentistry 2007; 20(6): 400-4.
39. Lessa FC, Aranha AM, Nogueira I, Giro EM, Hebling J, Costa CA. Toxicity of chlorhexidine on odontoblast-like cells. Journal of applied oral science : revista FOB 2010; 18(1): 50-8.
40. Zivanovic S, Li J, Davidson PM, Kit K. Physical, mechanical, and antibacterial properties of chitosan/PEO blend films. Biomacromolecules 2007; 8(5): 1505-10.
41. Schiffman JD, Schauer CL. Cross-linking chitosan nanofibers. Biomacromolecules 2007; 8(2): 594-601.
42. Yue IC, Poff J, Cortes ME, et al. A novel polymeric chlorhexidine delivery device for the treatment of periodontal disease. Biomaterials 2004; 25(17): 3743-50.
43. Fu Y, Kao WJ. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. Expert opinion on drug delivery 2010; 7(4): 429-44.

MONITORING ALL DAY KEEPS THE DOCTOR AWAY

By Lars Gallée and Jasper Kooijman

It is 4 pm and the attending nurse is walking her last round before passing over her responsibilities to the night shift. Although all patients are stable, she is overwhelmed by a strange gut feeling about one of her patients. There are no specific indications for clinical decline, but the thought of the patient not being observed until the next morning, does not quite feel right. The next day, a colleague informs the nurse that the patient had to be transferred to the Intensive Care Unit that night.

This is a well-known problem to many health care professionals, but if it is up to PhD-student and physician Mariska Weenk, it will soon be a problem of the past. Weenk is conducting her PhD-research studying the application of continuous monitoring to improve patient safety. This research is currently being carried out by the department of Surgery, Internal Medicine, Intensive Care and Radboud REshape Innovation Center of Radboudumc Nijmegen. Besides Weenk, the research team consists of Harry van Goor, Tom van de Belt (PhD), Bas Bredie (MD, PhD) and Lucien Engelen.

The initial concept of continuous monitoring came from the realisation that measuring a patient's vital signs three times a day is not always sufficient. From these three measurements the 'Modified Early Warning Score', or MEWS, is determined which represents the patient's clinical condition. This is however problematic as a sudden decline in a patient's condition may not be noticed immediately or even at all. Alternatively, when a patient is being continuously monitored, this decline in health can be noticed in an instant, making quicker interventions possible.

For this study, two devices are being used: the ViSi Mobile (VM) and the Healthpatch (HP). Both devices focus on the continuous registration of patients' vital signs. These measurements are then sent, via a highly secured network, to a central point. Both systems measure heart and respiratory rate, skin temperature and body position. VM can also measure blood pressure and oxygen saturation while HP can measure stress, which is a ratio between heart rate and heart rate variability. In terms of data collection, there is also a slight difference between both devices. HP sends its live data to a smartphone through a Bluetooth connection, which is then sent to a website. By logging into your account, the live information can be retrieved from literally anywhere on the planet. VM differs in that the system sends the live data directly to a computer via a secured WiFi network. With specially designed software, the measurements can then be directly read from the screen.

While HP lacks the ability to measure certain parameters, the device makes up for this by its size. It is a simple concept: one patch with an integrated sensor to measure the parameters. VM is more complex and a lot bigger, with multiple cables and patches attached to the patient's wrist, upper arm and chest. Additionally, the device has to be calibrated daily with a cuff in order to measure the patient's blood pressure.

The study was first started in the departments of Surgery and Internal Medicine in December 2014. "During the first phase of the study, the emphasis was put primarily on technical feasibility and determining the accuracy of both systems. We wanted to find out whether both systems' measurements differed from each other and - maybe even more important - whether their measurements differed from the nurses' measurements" says Weenk. "It is clear that the study is still in a very early stage; the second phase has just started this March (2015). In the second part of the study we focus mainly on the safety of patients and the general experience of both the patients and the nurses. Because of the differences between the devices in terms of their functioning and application on the patient's body, it is very important to make a clear assessment of the advantages and disadvantages of both systems."

Of course there is also a financial aspect to consider. The clinical implementation of the equipment means an investment for a hospital. The expectations are however that it will be very costeffective. The equipment itself is not expensive and can be broadly used to potentially prevent complications, which could lead to a significant decrease in Intensive Care admissions. Furthermore, it allows for earlier medical interventions, potentially resulting in a decrease in general hospitalisation. It could also provide a solution for nurses, as by reducing their workload more time can be spent with individual patients. "It is essentially a three-step goal: increasing patients' safety and comfort and reducing workload for the nurses."

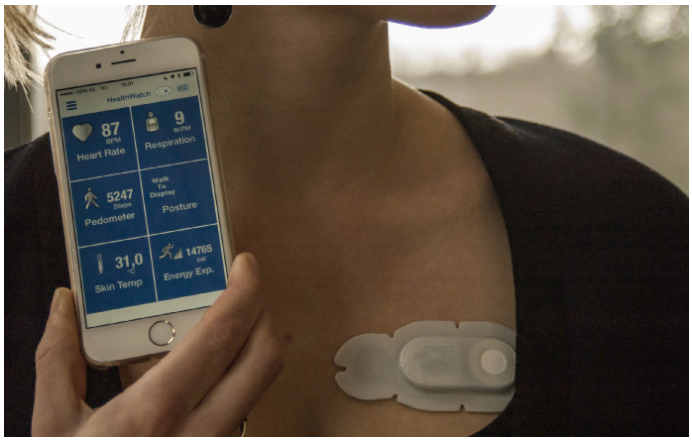

Figure 1 *Mariska Weenk showing the Healthpatch with accompanying smartphone application*

Even in this early stage of the study, several departments have expressed their interest in this new technology. "The division of Gastrointestinal and Liver Diseases has shown great interest, especially in the usage of continuous monitoring after endoscopy", according to Weenk.

It goes without saying that a lot of research has yet to be done before broad, clinical implementation of continuous monitoring will be possible. However, one thing that is certain is that the technology goes along perfectly with the current trend of healthcare bending more and more towards increased safety, efficiency, comfort and a more personal approach. Even at this early stage, patients have experienced an increased perception of safety, because of the knowledge that they are being continually monitored. With this trend in mind, the expectations are that the equipment will become even smaller and more user-friendly. Weenk has good faith in the wide deployment of the technology: "I certainly hope that within a year or two, this technology has been broadly implemented, both in hospitals as well as in patients' homes." A very promising and progressive vision if you ask us!

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