A Study on Oral Palliative Care

An exploratory study

Siri Flagestad Kvalheim

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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In memory of Inger Anne

Scientific environment

The work presented in this thesis was conducted during the years 2015-2019. The main supervisor was Professor Gunhild Vesterhus Strand and co-supervisor was Associate Professor Ileana Mihaela Costea.

Department of Clinical Dentistry

Faculty of Medicine

University of Bergen, Norway

Collaborators:

Sunniva Centre for Palliative Care, Haraldsplass Deaconess Hospital, Bergen

Palliative Department, Red Cross Nursing Home, Bergen

Centre for Geriatric- and Nursing Home medicine (SEFAS), UiB, Bergen

Regional Centre of Excellence for Palliative Care, Western Norway

Department of Pathology, Haukeland University Hospital, Bergen, and

Tannteam Dental Clinic, Nesttun

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Abbreviations

EBM: Evidence based medicine

EBHC: Evidence based health care

ECOG: Eastern Cooperative Oncology Group

EPCRC: European Palliative Care Research Collaborative

ESAS: Edmonton Symptom Assessment System

IHC: Immunohistochemistry

IAHPC: International Association for Hospice and Palliative Care

IV: Intravenous

KPS: Karnofsky Performance Status

LCP: Liverpool Care Pathway

LTC: long-time-care

MIK: Medication induced dry mouth

NHS: National Health Service

RCT: Randomized Controlled Trial

REC: Regional Ethical Committee

RNHBM: Reconstructed Normal Human Buccal Mucosa

SGD: Salivary Gland Dysfunction

WMA: World Medical Association

Abstract

Objective: Xerostomia is a substantial problem for a majority of patients in palliative care. Guidelines that exist for palliative care are mainly based on tradition and long-time experience. Scientific evidence is sparse. Consequently, one of the agents used for lubrication, glycerol, is recommended in some countries, while not recommended in others. Presently, little is known about the effects of different procedures for oral palliative care.

Aim: The overall aim was to study procedures and oral care products with the aspiration of contributing in some measure to the body of knowledge within the field of oral palliative care and its future guidelines.

Material and methods: A questionnaire study was conducted to explore circumstances surrounding procedures and knowledge regarding oral palliative care in Norwegian healthcare institutions. An in vitro study on reconstructed human oral mucosa was used to explore biological, dose-dependent effects of glycerol. Finally, the effectiveness of three different oral moisturizers were compared in a randomized controlled trial (RCT) in palliative care patients suffering from xerostomia.

Results: The questionnaire study revealed that a plethora of different procedures for oral palliative care exist and that 25 % do not have oral palliative care procedures at all. The laboratory study showed that glycerol in concentrations of 42.5% and over led to an increase in cell proliferation and apoptosis, but had no effect on tissue integrity. In the RCT, 17% glycerol had the best effect directly after application, but no effect after two hours. The two other products had long-lasting effect, but were not preferred by the patients.

Conclusions: There is an obvious need for awareness about a standardisation of oral palliative care. Glycerol does not seem to harm the mucosa in low concentrations, but lacks long-term effect. Other products may be more effective, but taste and consistency must be modified to suit the patient group.

List of publications

I. **Kvalheim SF**, Strand GV, Husebø BS, Marthinussen MC. End-of-life palliative oral care in Norwegian health institutions. An exploratory study. Gerodontology. 2016 Dec;33(4):522-529.

II. **Kvalheim SF**, Xenaki V, Kvalheim A, Lie SA, Marthinussen MC, Strand GV, Costea DE. Effect of glycerol on reconstructed human oral mucosa. Eur J Oral Sci. 2019 Feb;127(1):19-26.

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Contents

1. Introduction

1.1 General background

The majority of seriously ill and dying patients have problems with xerostomia, the subjective feeling of dry mouth (1, 2). The dry mouth problem may be caused by medication, treatments or as a direct result of the mortal condition (3-5). This condition can lead to oral pain, dysphagia, speech disturbances, loss of appetite, dehydration and malnutrition, thus affecting the disease negatively and contributing to reduced quality of life (6-9). Until now, there is no strong evidence that any topical therapy is effective in relieving the symptom of dry mouth (10). In 2017 82% of deaths in Norway occurred in an institution: 32% in hospitals and 50% in nursing homes (11).

1.2 Definition of palliative care

Palliative medicine is the term used for the medical specialty area, whereas Palliative Care is used for the field as a whole (12). When palliative medicine was approved as a specialty in the United Kingdom in 1987, a definition was made specifically aimed for medicine: “Palliative medicine is the study and management of patients with active, progressive, far advanced disease, for whom the prognosis is limited and the focus of care is quality of life” (13). The term terminal is often imprecisely used synonymously with palliative, but primarily the term terminal describes the last few hours or days before death (14).

Several definitions for palliative care have been proposed. The first WHO definition, from 1990, emphasized its relevance to patients who did not respond to curative therapy (15). This statement could be interpreted as relegating palliative care to the last stages of care:

“Palliative care is the active, total care of patients with progressive, far advanced disease and limited life expectancy whose disease is not responsive to curative

treatment. It refers to the control of pain and of other symptoms as well as the treatment of social, psychological, and spiritual problems". (WHO, 1990) (15).

Today, there is a wider recognition that the principles of palliative care should be applied as early as possible in the course of any chronic, ultimately fatal illness (16):

"Palliative care is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial, and spiritual". (WHO, 2002) (17).

WHO's latest definition has gradually become quite widespread, but it describes palliative care as an approach. The European Association for Palliative Care (EAPC) sees palliative care not only as an approach, but also as a discipline in its own right. That is probably one of the reasons why EAPC has a definition of palliative care much like the first WHO definition (18).

International Association for Hospice and Palliative Care (IAHPC) has just developed a new (2018) definition of palliative care that is receiving broad support:

"Palliative care is the active holistic care of individuals across all ages with serious health-related suffering due to severe illness, and especially of those near the end of life. It aims to improve the quality of life of patients, their families and their caregivers" (19).

1.3 History of palliative care

Until the end of the 19th century, healthcare services consisted primarily of care and relief. Health institutions were often linked and located to churches and monasteries (Fig. 1) (20). With the introduction of anaesthetic methods from 1856 (21), X-rays from around 1900s (22) and antibiotics from the 1950s (23), medicine faced new opportunities and the focus was changed to treatment and therapy. As a result, major clinical advances characterized the 1950s. The goal was to cure everyone. Those who

could not be cured were often perceived as a defeat for the therapist. Care and relief lost much of their status (20). Parallel to the modernization that took place in medicine, a scepticism developed. The philosopher Ivan Illich went to a frontal attack against modern medicine, which he believed had done more harm than good. Illich claimed that death and suffering were removed from modern medicine (24).

Fig. 1. *Healthcare at a French Hospice in the 18th century, Hospice des Dames du Calvaire, Marseille. Photo: Archives départementales. (Reprinted with permission.)*

Cicely Saunders (1918-2005) is considered the founder of the modern palliative care movement. Her interest in palliative care and pain control developed early. She saw that particularly better pain control was needed (18). To gain acceptance for her ideas, she graduated as a physician and started planning an inpatient unit for dying patients. St Christopher's Hospice, London, was opened in 1967 and was the first modern research and teaching medical unit linking expert pain and symptom control, compassionate care, teaching and clinical research, pioneering the field of palliative medicine (25).

The history of hospice philosophy and palliative care is complex and embraces humanistic ideas, medicine, public involvement and academic subjects. The entrepreneurs of the hospice movement often had a Christian conviction. Recent,

non-denominational approaches have promoted other systems and professionalization. However, many institutions today are still based on earlier work by Christian organizations, including continuation of deacon departments at hospitals and homecare for patients dying at home. The development of the field of palliative care has been a process evolving from voluntary work to a specific field of medicine (20). From 1975, the term "palliative care" was applied internationally. In many countries, including Norway, physicians were not very visible in the palliative care field until the end of the 1980s and the beginning of the 1990s (12). There was a debate whether care for the dying was a task for the health services. In 1988, the European Association for Palliative Care was established (18). In the same era, a collaboration was established between different professionals in Norway, with nurses, physicians, priests and social workers. The degree of interprofessional collaboration needed to care for seriously ill and dying patients is higher than in most other fields within the health services (12).

History of palliative care in Norway

Priority has been given to palliative care in three public health recommendations, from 1984 (26), 1987 (27) and 1997 (28), respectively.

The first palliative care unit in Norway was opened at the university hospital in Trondheim in 1993 (29). Norway's first palliative department in a nursing home was opened at Bergen Red Cross Nursing Home in the year 2000 (30). In 2011, palliative medicine was established as a separate discipline (formal competence field) on a trial basis in Norway (31).

The Dental Health Act ("Lov om Tannhelsetjenester") from 1984 (32) ensures that dental services are available to the entire population. It also specifically provides outreaching and regular treatment for some prioritized groups, including patients in need of oral palliative care.

1.4 Standardized care approaches and assessment tools in palliative care

1.4.1 Standardized care approaches for end of life

Since the mid-1980s, standardized care pathways have been an important tool in clinical improvement work (33). The purpose of standardized processes is to create security and predictability, ensure high professional quality and contribute to good cooperation, efficient resource utilization and measurable results (34). Internationally, there are a number of care plans for the final phase of life. The most widely known and used is the Liverpool Care Pathway for Care of the dying Patient (LCP) (35). The plan was developed by the Royal Liverpool University Hospital and Marie Curie Hospice in Liverpool for their own use, but eventually spread both nationally and internationally. The plan is intended for the short period from the patient is defined as dying until the first few hours after death. In 2009, LCP received negative news attention, claiming that the plan was used uncritically and could accelerate patients' death (36). The criticism led the British government to appoint an independent commission, which delivered its report in July 2013, the Neuberger review (37). The review report concluded that LCP was based on sound ethical principles and contributed to a good and peaceful death when the plan was used according to the intention. However, the report also concluded that poor implementation of the LCP had led to unfortunate situations for dying patients in hospitals, and the report recommended that the LCP should be phased out within a year (37, 38). The phasing out met with a lot of criticism and disagreement and was seen by many as a pure political decision (33, 38). In Norway, a continued but revised plan has taken over for LCP (39). The revised plan has been criticized for not being suitable for people with dementia and for many nursing home residents in general, and the critics believe the plan should rather have been replaced with competence-raising measures (30, 40, 41). Supporters of the plan believe it is a suitable tool and that it has led to greater awareness and expertise in palliative care (33, 40, 42, 43).

1.4.2 Assessment of subjective symptoms in palliative care

Palliative care is intended to relieve troublesome subjective symptoms. Studies have shown that systematic assessment of symptoms is an important prerequisite for optimal relief (44). To that end, the Edmonton Symptom Assessment System (ESAS) was designed to perform repeated measurements of symptom intensity with minimal inconvenience to the patient (45). It is now one of the most used forms for self-reporting of symptoms within palliative medicine (46). The original version of the ESAS form covers seven of the most common symptoms of long-term cancer: pain, fatigue, nausea, depression, anxiety, loss of appetite and heavy breathing. In addition, general well-being is assessed and the form has one open category where the patient can enter a specific symptom (45). The symptom intensity is indicated on an 11-point numeric scale. When the form was translated and used in Norway at the university hospital in Trondheim (St. Olavs hospital) in 1999, the open category was left out while xerostomia and motion pain were included in the form. This version, known as Trondheim Palliative Assessment Tool (T-PAT), was widely used in Norway and was recommended and included in the national palliative action program (47). ESAS has later been revised, and the revised version ESAS-r is now internationally used and recommended as standard palliative care assessment tool in Norway (48, 49). This revised version does not include dry mouth, but has an open category where this may be added.

In medicine, performance status is an attempt to quantify the patient's function and activities of daily life. The WHO (ECOG) Performance Status and the Karnofsky Performance Status (KPS) Scale are two widely used methods to assess the functional status of a patient. Both scales have been in the public domain for many years as ways to classify a patient according to their functional impairment, compare the effectiveness of therapies, and assess the prognosis of a patient. The Karnofsky Performance Status Scale (0-100) is one of the most commonly used. Low KPS status is one of the best prognostic indices, especially in cancer, indicating limited remaining life time (50, 51). WHO status is coarser and easier to use on a daily basis. WHO status is for example used in the selection of patients for certain types of

treatment. It is also a part of the ESAS form. There are comparisons of the WHO and the Karnofsky performance status scales (Fig. 2) (52). Since the scales have different wording, there is no completely linear relationship between them.

Karnofsky Status	Karnofsky Grade	WHO Grade	WHO Status
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction
Able to carry on normal activities. Minor signs or symptoms of disease	90	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
Normal activity with effort	80	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
Care for self. Unable to carry on normal activity or to do active work	70	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires occasional assistance, but able to care for most of his needs	60	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires considerable assistance and frequent medical care	50	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
Disabled. Requires special care and assistance	40	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
Severely disabled. Hospitalisation indicated though death not imminent	30	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Very sick. Hospitalisation necessary. Active supportive treatment necessary	20	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Moribund	10	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Dead	0	5	Dead

Fig. 2. *Relationship between Karnofsky and WHO status.*

1.5 Dry mouth in palliative care patients

Saliva protects teeth and oral mucosa; it facilitates articulation of speech, is important for mastication and deglutition and is of significance for both oral homeostasis and for maintaining oral health (53-55).

The saliva secretion is exclusively under the control of autonomous reflexes. Saliva is mainly produced by the parotid, submandibular and sublingual glands. Within the glands, the acinar cells are responsible for the volume of saliva secreted, and the duct cells are responsible for the composition of saliva (56). Unlike the autonomic nervous system in other places of the body, sympathetic and parasympathetic response in the salivary glands are not mutually contradictory (57). Increased activity in the sympathetic fibres of the glands leads to a slight increase in the rate of excretion and a more viscous quality of saliva. Increased parasympathetic activity, on the other hand, gives a large increase in thin-flowing secretion. Volume and consistency of saliva are thus dependent on the balance between the activity of the sympathetic and the parasympathetic nerve fibres (56, 58).

1.5.1 Definitions of 'dry mouth'

Dry mouth is a generic term that can include different conditions:

Xerostomia is defined as the subjective sensation of dry mouth (59).

Hyposalivation is defined as objectively and measured reduced salivation (59). Unstimulated flow rate of <0.1 ml/min is considered evidence of hyposalivation, defined as the objective finding of a reduced salivary flow rate (60).

Salivary Gland Dysfunction (SGD) is defined as any alterations in the qualitative or quantitative output of saliva caused by an increase (hyperfunction) or decrease (hypofunction) in salivary output (61, 62).

1.5.2 Causes of xerostomia in palliative care patients

Xerostomia occurs when the salivary flow rate is less than the rate of fluid loss from the mouth by swallowing, evaporation and absorption of water through the oral mucosa. Saliva in the residual volume is present as a thin film which varies in thickness with site. The hard palate has the thinnest film and when it is < 10µm thick, evaporation may rapidly decrease it to zero. This is generally associated with reduced secretion from the soft palate's minor glands. Thus, xerostomia appears to be due, not only to a complete absence of oral fluid, but to localized areas of mucosal dryness, notably in the palate (63).

Diseases causing oral dryness

Several diseases are associated with salivary gland hypofunction (55, 64). In autoimmune diseases, such as Sjögren's syndrome, Rheumatoid Arthritis and Systemic Lupus Erythematosus, salivary gland dysfunction is largely related to structural changes in the salivary glands (65-68). In endocrine and metabolic disorders, like diabetes mellitus and thyroiditis, the oral problems are mainly related to pathophysiological changes that affect the formation of saliva (69, 70). In addition, there are a range of diseases that affect the autonomic outflow pathway involving the salivary gland innervation, the central nervous system and the salivation centre (71-73).

Side effects of head and neck irradiation treatment for cancer

Most patients requiring specialist palliative care are cancer patients (74, 75). Xerostomia is extremely common in patients who have received radiotherapy to the head and neck region, with a prevalence of up 78-82% in advanced oncology groups (76, 77). In patients who receive chemotherapy, the prevalence of xerostomia has been estimated to be 50 % (76).

Side effects of medication and polypharmacy

The effect of medication on the saliva secretion is complex. Medications may simultaneously interact with the salivary reflex at different sites, on the central nervous system and/or on the neuroglandular junction on muscarinic, α- and

βadrenergic receptors and on certain peptidergic receptors (5, 78). Polypharmacy increases the risk of interactions and xerostomia (79, 80). It is uncertain to what extent medications induce xerostomia, most studies report the subjective complaint and there are only a few studies that measure objective changes in salivary flow (81).

Opioid analgesics, sedatives, neuroleptics and anticholinergics are drugs that are often used in palliative care and continued into or added in the terminal phase (82, 83). In a longer time-perspective, drugs for neuropathic pain are also often used, i.e. pregabalin, gabapentin and amitriptyline. These cannot be applied when the patient is no longer able to swallow tablets. Octreotide can be used with anticholinergics against ileus. Octreotide is given parenterally and can be given into the dying phase. Proton pump inhibitors are also used by some patients, especially those using corticosteroids and can cause dry mouth (79). The proton pump inhibitors are discontinued before the dying phase.

Dehydration and xerostomia

Dehydration is a common cause of xerostomia among palliative patients. Common causes are insufficient intake of fluid, fever and medications, which affect the regulation of the salt and water balance in the body (84, 85). Mouth breathing has also been found to be a common cause of mucosal dehydration and a cause of xerostomia (86). Treating dehydration in palliative patients is challenging in view of complex physical, moral, ethical and cultural factors (87).

1.5.3 Concequences of xerostomia in palliative patients

Reduced Lubrication of oral surfaces

The mucins in saliva are highly glycosylated proteins that form a hydrophilic network (88). This acellular film of mucin and water moistens and lubricates oral surfaces and gives saliva its texture and viscosity (89). The lubricating properties are important in the protection of mucous membranes, for mastication and deglutition and they

facilitate articulation of speech (53-55, 90). In seriously ill and palliative patients, the lack of lubrication by saliva may cause soreness, speech and deglutition problems. (3, 6, 61, 84, 91, 92).

Decreased buffer capacity and protection of teeth

The buffer properties of saliva consist of bicarbonate, phosphate and protein systems. These are important in the protection of teeth against acid attacks, but the buffer properties also have an important role in promotion and maintenance of a balanced oral microbiota (93, 94). Lipids in enamel pellicles are shown to protect the enamel surface against acid by retarding the lactic acid diffusion (95, 96). Persistent and severe hyposalivation may lead to increased caries activity with lesions on cervical, incisal and cuspal tooth surfaces (97, 98).

Lack of salivary clearance

Containing substantial amounts of water, saliva has the ability to dilute and remove food substances, desquamated epithelial cells and microorganisms from the oral cavity (99-101). Saliva secretion and swallowing thus promote removal of bacteria and play an important role in balancing the microbiota (102, 103). In severely ill people, dry mouth will cause bacteria and food residues to accumulate in the mouth, causing a change the microbiological balance and thus discomfort and halitosis (2, 7, 9, 91, 92, 104).

Antimicrobial actions and healing properties

The oral microbiota in healthy individuals is very diverse; more than 700 species have been identified in the oral cavity (105) and the oral cavity contains many niches for microbiological colonization (106). Saliva is important for both oral homeostasis and symbiosis (107), and is well known for its important role in maintaining oral health (108). Saliva provides antimicrobial activity through numerous proteins and peptides, including mucins, lactoferrin, lysozyme, lactoperoxidase, statherin, histatins and secretory immunoglobulin A, and promotes wound healing in several ways (55, 107, 109). A reason why wounds grow faster in the oral cavity than on the skin is that saliva creates a humid environment that increases survival and functioning of

inflammatory cells that are crucial for wound healing (110). An in vitro study has shown that saliva initiates the formation of pro-inflammatory macrophages, which are important for renovation and defence functions (111). Lack of saliva will destroy the microbiological equilibrium and combined with soreness and cracks and impaired immune system, the condition of oral dryness will often cause infections (2, 6, 7, 9, 91, 92, 112, 113).

Fig. 3 *The problem with oral dryness became encompassing and drew attention away from what you really wanted to spend your last time on, said Inger Anne.*
Photo of Inger Anne Bolme, who died of cancer in 2012, and her son Ruben, taken by Espen Bakken, Adresseavisen. (Printed with permission from the family and Adresseavisen.)

Impaired taste and digestion

Through direct mechanisms such as molecular, enzymatic and dilution, saliva can modify release or aroma compounds. Aroma perception is an important factor for the acceptance of food and thus for appetite (114). Food intake induces both mechanical, olfactory and chemical stimuli via neural reflexes, resulting in an increased salivation

(90). Saliva plays an important role in the digestive processes; taste, breakdown of foods, masticatory function, bolus formation and deglutition (115). Lack of appetite, and hence nutritional intake in palliative patients, becomes a vicious circle where symptoms worsen and dehydration increases (7, 91, 116). Lack of liquid and degradative enzymes often lead to constipation (117).

1.5.4 Treatments

Saliva substitutes

Saliva substitutes are available in a variety of forms, including sprays, gels and lozenges (118, 119). They are often based on mucins, methylcellulose or modified cooking oils (10, 119). A Cochrane review from 2011 concluded that there is no strong evidence that any topical therapy is effective in relieving dry mouth (10).

Lemon and glycerine has been used for about 70 years as oral moisturizer for patients who experience xerostomia (120). However, lemon juice increases salivation and it has been claimed this can lead to a reflex exhaustion of the salivary glands over time (121). In addition, it has been claimed in several studies that glycerol`s absorption of water actually causes drying the mouth rather than lubricating it (122-124).

Saliva stimulants

A number of different stimuli may increase salivary flow (53). These can be triggered using chewing gums, lozenges, and by adding ascorbic and citric acid (118). For palliative patients it may be a problem to dissolve lozenges due to a total lack of saliva. Also, chewing gum requires adequate chewing force, which may not be available.

Pilocarpine is a muscarinic receptor agonist, which has been shown to be an effective treatment for xerostomia (119, 125). Its use, however, involves some undesirable side effects, such as sweating, headache, urinary frequency and vasodilation (126).

Salivary stimulants are only of benefit for those with remaining functional salivary gland tissue and a mild xerostomia (53).

Artificial hydration

Artificial hydration is defined as providing solutions thorough non-normal routes, such as intravenously, subcutaneously, dermally, rectally or as a component of enteral nutrition or parenteral nutrition. Studies show conflicting results in terms of advantages and disadvantages (127). The advantage of artificial hydration is, among other things, that it prevents the accumulation of drugs and reduces fatigue, dizziness and reduced awareness (128). Disadvantages of artificial hydration include painful edema and prolonging the death process (129).

Other approaches

Hyperbaric treatment has been showed to result in significant decrease in xerostomia in irradiated head and neck cancer patients (130, 131). An electro stimulating device has been tried out (132), yet the experience and evidence is insufficient. Acupuncture has been tried as a treatment against xerostomia, but so far, there is insufficient evidence regarding the effect (133). Recent research attempts have been made to solve problems with xerostomia with nanotechnology (134). So far, it has not yielded results that can be implemented in clinical use.

1.6 Oral palliative care

Guidelines for oral palliative care

In the wake of the development of the field of palliative medicine, some countries have developed national guidelines for oral palliative care (135-140). The guidelines are based on available evidence, but mainly on health care personnel`s experience and tradition. The main steps of the recommendations are fairly similar: Oral care is initiated by oral assessment using a recognized grading system (141), followed by care of the oral cavity consisting of cleaning teeth, gums/mucosa and lubrication of lips and mucosa (139). Although the main features of the procedures have much in common, the recommendations vary between the countries, especially when it comes to the oral care products. While the Norwegian guideline recommends glycerol as moisturizer, the use of glycerol in oral care procedures is discouraged in several other countries and in scientific literature, due to its possibly desiccating effect (121, 138).

If this is correct, it will adversely affect thousands of dying patients, resulting in chronic and increasing oral dryness.

Oral palliative care in Norway

Institutionalized, seriously ill and dying patients are entitled to free examination and treatment by a public dental officer – either a dentist or a dental hygienist (32). Patients living at home are entitled to the same, provided that they receive home care. Patient's and User's rights Act ("Lov om pasient- og brukerrettigheter") defines similar rights for patients in Norwegian health institutions (142). Daily care should be provided by nursing staff in the hospital or nursing home. Oral care is a legally required part of daily care.

2. Aims

2.1 Overall aim

To study some oral procedures in Norwegian health care institutions and to evaluate a selected sample of oral care products related to xerostomia in palliative care patients.

2.2 Spesific aims

Without intervention:

- Identify procedures for oral palliative care in Norwegian Health Institutions
- Identify oral care products applied in oral palliative care in Norway
- Explore knowledge about oral palliative care among Norwegian health care personnel

With intervention:

- Investigate the effect of glycerol in three different concentrations on cells and cell layers in an oral mucosa model
- Investigate the subjective effect of three different oral moisturizers on xerostomia, discomfort/pain, speech problems, taste, application method and preference in palliative care patients

3. Material and methods

The materials and methods used in these studies (Paper I, II and III) are extensively described in the respective papers. A brief summary of the materials and methods used follows:

3.1 Material

Study I: A selection of hospitals (n=19) and nursing homes (n=57) participated.

Study II: A total of 96 samples of Reconstructed Normal Human Buccal Mucosa (RNHBM) matured from biopsies from eight donors were used (Fig. 4).

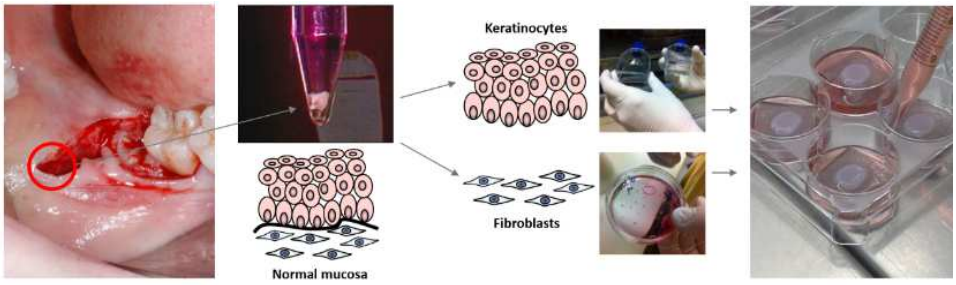

Fig. 4. *Schematic illustration of reconstruction procedure, from biopsy, via cultivation of 2D cell cultures, keratinocytes and fibroblasts to the 3D RNHBM samples.*
Photoes/Illustration: S. Kvalheim/D.E. Costea

Study III: Thirty palliative patients with xerostomia met the inclusion criteria and were willing to participate.

3.2 Methods

Study I: Epidemiological method

The geographically representative and randomly selected participating hospitals and nursing homes were asked to complete a questionnaire that included three closed- and three open-ended questions about oral care for terminal patients. If procedures existed, the respondents were asked to enclose or describe them. The charge nurse or deputy was asked to complete the questionnaire.

Study II: In vitro, cell culture experiment

The 96 RNHBM samples were exposed to 17%, 42.5% or 85% glycerol, or to distilled H_2O (control). After 24 hours, the samples were paraffin embedded. From each of these, 384 sections were made available for analysis by either immunohistochemistry (IHC) to measure proliferation, apoptosis and cell-integrity, or histomorphometry, to measure epithelial thickness (Fig. 5.).

Fig. 5. *Illustration of procedure for exposure and analysis.*
Illustration/photos: S. Kvalheim.

Study III: In vivo RCT study

The patients were exposed to a 17% solution of glycerol, Aequasyal® and Salient®. Each of the three products was applied after morning routine care and breakfast for three days. Each intervention was initiated with an oral care procedure (Fig. 6). The patients were blinded to the type of product applied. The order in which the products was applied, was determined by block randomization prior to interventions. Measures of subjective xerostomia, discomfort/pain and speech-problems were recorded before intervention, right after and two hours after application of the product. In addition, evaluation of taste, application method and patient preference of the products used were recorded at the two latter points in time. After all procedures were completed, the patients were asked to comment the products and the procedures freely.

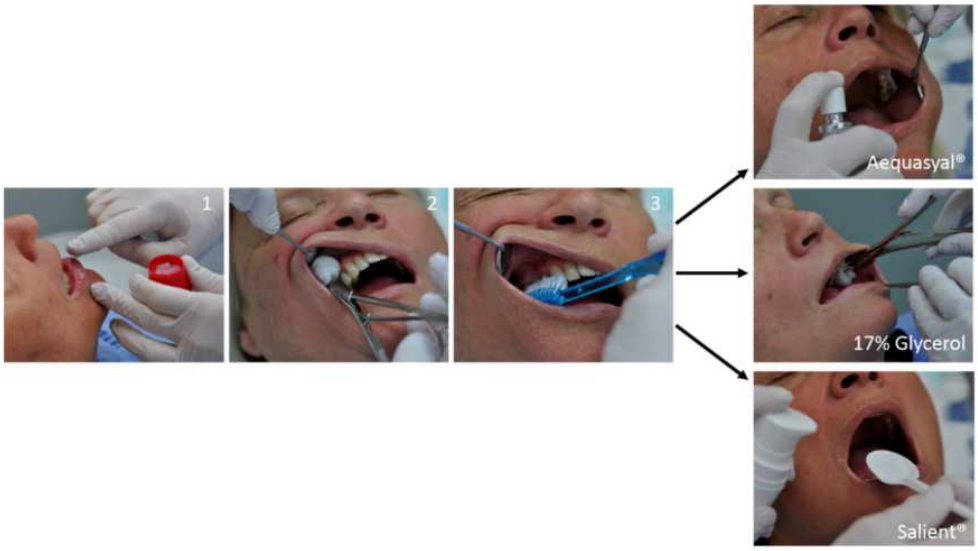

Fig. 6. *Illustration of the initial care procedure and the following intervention procedures with the products Aequasyal®, 17% glycerol solution and Salient®.*
Illustration/Photo: S. Kvalheim, K. Reisegg

3.3 Statistical analyses

The table below (Table 1) gives an overview of the statistical methods used.

Statistical methods	**Paper I**	**Paper II**	**Paper III**
Descriptive statistics	●	●	●
Fisher`s exact test	●		
Linear mixed effects model		●	
Intraclass correlation (ICC)		●	
Likelihood ratio test		●	
Ordinal logistic regression			●
Odds ratios (ORs)			●
Chi test			●

Table 1. *Statistical methods used in the analysis.*

Study I

Frequencies of the responses were counted. Hospitals and nursing homes were compared using Fisher`s exact test. The analyses were performed with SPSS 20 (IBM Corp, IBM Statistics for Windows, Version 20.0, Armonk, NY). A 5% significance level was chosen for all analysis.

Study II

For comparisons of the parameters across 17%, 42.5% or 85% concentrations of glycerol in the in vitro study, linear mixed effects models were applied. In these models, for each outcome, concentration was entered as a categorical fixed effect. Donor was included and controlled for in the model as a random effect accounting for the possible correlation between samples from the same donor. The main results, based on the mixed models, were presented as estimated marginal mean values and

mean differences, with 95% CI. Analysis were performed using the statistical package STATA version 15 (Stata, College Station, TX, USA).

Study III

For the categorical variables, percentages and frequencies were reported. To analyse differences between three oral care products, ordinal logistic regression was applied, with robust variance estimates to adjust the p-values for possible correlation between the nine repeated observations for each individual. Changes from the baseline measures for each of the oral care products and differences between the products right after and two hours after application were reported as odds ratios (ORs). To test if the distribution of preferred product was uniform for the three products, a chi-squared test was applied. The statistical analyses were performed using Stata (version 15, TX, USA). P-values less than 0.05 were considered statistically significant.

4. Summary of Results

4.1 Study I

Response rate

The response rate was 84 % for hospitals and 79 % for nursing homes.

Procedures for oral palliative care

Twenty-five per cent of the responding institutions had no procedures for oral palliative care. Twenty-one different procedures were identified. A great variety of products is used for lubrication; the most common one being different concentrations of glycerol – 98% of which were above 30%.

Attitudes and knowledge of oral palliative care

An overall of 39 % reported that they had insufficient knowledge of oral palliative care. More nursing homes (56%) than hospitals (25%) answered they did not recognize that oral problems were of particular significance for palliative care patients.

4.2 Study II

Biological effects of glycerol on oral mucosa

Epithelial thickness, proliferation and apoptosis were significantly increased by exposure to 42.5 % and 85 % glycerol. No significant difference in apoptosis and proliferation was found between controls and RNHBM. Cadherins, which are cell adhesion molecules indicating tissue integrity, were not significantly affected by exposure to any of the concentrations of glycerol tested. Glycerol affected tissue homeostasis (cell proliferation and apoptotic cell death), but not tissue integrity of RNHBM at glycerol concentration above 42.5 %.

4.3 Study III

Response rate

The response rate was 100% (no missing data).

Subjective effects of the three different oral moisturizers

Directly after application, compared with baseline, all products had a significant effect on the outcome variables: xerostomia, pain/discomfort and speech problems. A 17% concentration of glycerol provided the best relief.

After two hours, the glycerol solution had no significant effect on the same variables, whereas the effects of Aequasyal® and Salient® were maintained.

Taste and texture

The taste of Aequasyal® was disliked by 77% of the responders. Regarding Salient®, 87% found the taste neutral, whereas all respondents found the taste of the glycerol solution agreeable or neutral.

The texture of Salient® was found to be disagreeable and sticky by 60%.

Application method

Most respondents found the use of a soaked gauze pad (glycerol solution) or a spray (Aequasyal®) preferable to dispensing by means of a spoon Salient®.

Patient preference

The majority of patients (63%) preferred the glycerol solution.

5. Discussion

5.1 Methodological considerations

5.1.1 Internal and external validity of the studies

The validity of a study can be defined as the extent to which the inference drawn from the study is warranted when account is taken of the study methods, the representativeness of the study sample and the nature of the population from which it is drawn (143). If internal validity is acceptable, it is valid for the sample studied, whereas external validity refers to the degree to which the effect of the treatment can be generalized to other patients and other settings than the ones investigated in the experiment (144, 145). In the following sections some of the most important aspects of internal and external validity are discussed.

Study I - The questionnaire study

Questionnaire studies often have the advantage that they are not very resource-intensive and can be given to relatively large samples. This questionnaire study was sent to 76 randomly selected institutions with a geographical distribution throughout all 19 counties of Norway, in order to assure that the results could be regarded as representative and generalizable for the whole country.

A disadvantage of the questionnaire method is that misunderstandings may be difficult to resolve. Choosing and formulating questions is crucial to the internal validity. Ideally, several issues should express nuances of a specific construct (145). However, too comprehensive questionnaires will often achieve a lower response rate. Even though a more comprehensive questionnaire with more items might produce more nuanced responses, for the purposes of this thesis, the important point was to ascertain if procedures did exist and what they entailed. Thus, in the present questionnaire study, emphasis was placed on using few and specific questions: three closed-ended and three open-ended questions, keeping each issue concrete and with

less risk of misunderstanding. None of the respondents in this study expressed a need for clarification.

Another disadvantage of questionnaire surveys is that the response rate may be low, often less than 50%. A low response rate will reduce both internal and external validity because responders may differ from non-responders, which may increase the risk of bias. Studies have shown that use of reminders may improve the response rate (146). The present response rate was 80% (79% for nursing homes and 84% for hospitals). This is a higher number than usual for this type of studies, which strengthens the validity of the results.

Study II - The in vitro study

In order to study effects of moisturizers at the cellular level, without having to involve patients or laboratory animals, functionally relevant, experimental models are needed. The conventional cell culture models, in which cells grow two-dimensionally in monolayers, lack the interactions with other cells by which they would normally be surrounded in real life (147). The reconstructed normal human buccal mucosa model (RNHBM) has been developed by Dr. Costea, University of Bergen and was originally developed for oral cancer research (148, 149). Advantages of the RNHBM are that it allows epithelial-mesenchymal interactions and offers a great flexibility for study design as each of its constituents can be modified. RNHBM also involves less ethical concerns than testing directly on the mucosa of living individuals, it is reproducible and can be standardized. Limitations of the RNHBM are the restricted life span, the lack of vascular and immunocompetent components and the fact that it is technically and financially demanding.

Because immunohistochemistry (IHC) is an extremely expensive and time-consuming procedure, only three randomized RNHBM samples from each donor/concentration were used. The fact that not all samples were used for these IHC analyses led to some reduction of statistical strength. Nevertheless, statistically analysis resulted in statistically significant results.

Another advantage of the model system is the possibility of producing a large number of RNHBM samples from one donor, often more than 20. From the six accepted donors a total of 96 RNHBM samples were obtained. The samples were considered at two levels; as donor and as individual RNHBM samples. Variability of the outcome can therefore be thought of as either being within the RNHBM samples from the same donor or between RNHBM samples in general. For that reason, the donor was included and controlled for in the model as a random effect accounting for the possible correlation between samples from the same donor. Concentration was entered as a categorical fixed effect.

Possible sources of bias were primarily technology-sensitive factors, such as the viability of the cells and the time slot during manufacture. During maturation, the size could vary somewhat, which obviously affected the exposure surface. Due to the concave centre part of the RNHBM sample and the potential for the outer parts not being exposed, all measurements were made in a standardized distance from the centre.

Study III - The RCT

The RCT is the most scientifically rigorous method of hypothesis testing available and is regarded as the gold standard trial for evaluating the effectiveness of interventions (150). Empirical evidence indicates that inaccurate or inadequate reporting of information is associated with biased estimates of treatment effect (151). CONSORT 2010 Guidelines have therefore been used as the basis for this RCT.

On the basis of ethical, financial, practical and resource-intensive considerations in relation to the patient group (152), sample size is relatively small and only a few variables were explored. Still, the sample size was sufficient for adequate statistical analysis. In order to avoid having to include unnecessarily large numbers of participants, a crossover design was applied in this RCT. Each case was self-matched by serving as its own control (153). With the cross-over design performance bias,

systematic differences in the provided care, and detection bias, systematic differences between comparison groups, were avoided (154).

The external validity of this study has some obvious limitations. Healthier individuals may perceive their oral dryness, speech problems and discomfort as less invasive than severely ill, palliative patients and may, to a greater extent, be able to prevent their problems themselves when needed. The results of this study are thus not necessarily generalizable for other types of xerostomia patients.

A block randomisation, with blocks of six, was used to ensure a balance in the number of patients allocated to each of the sequences in the trial (150). Studies have shown that treatment effects have been exaggerated in trials in which allocation treatment have not been concealed (155). In this RCT, allocation concealment was completed by one of the co-authors who was not responsible for the recruitment.

Blinding means that the nature of the treatment is not known by the parties involved in the RCT experiment. The object of blinding is to reduce the risk of bias, best accomplished with a double-blind study, i.e. that both patient and researcher are thus blinded. However, for a number of reasons a double-blind method cannot always be applied in clinical studies, and a single-blind method must then be applied. (144). In our context, the three oral moisturizers were presented in neutral containers without label. Both participants, data collectors and data evaluators were kept ignorant of the assigned treatment. The dentist who carried out the intervention could not be similarly blinded because of differences in application method, which sufficed to identify the treatment.

A significant element of uncertainty was related to the fact that while only very ill patients were recruited, they would hopefully survive and remain in an unchanged health condition throughout the entire study period. That no participants withdrew from the study or were lost to follow-up, helped increase internal validity and avoid attrition bias, i.e. biased occurrence and handling of deviations from protocol and loss to follow up (156). The reasons for this was primarily due to knowledgeable and professional help with enrolling suitable patients, but also obviously a great deal of

luck. In such patients, the health condition can change quickly and radically from one day to the next (157).

Another source of error was that it was not possible to standardize the interventions within the ethical and practical framework. Our study could not interfere with the procedures of oral care of the institution, although these were used only to a limited extent during the intervention period. However, in theory, the hospital`s and patient`s own oral care procedures may have affected the effect of the interventions. The study intervention was applied right after breakfast and morning routine care. For ethical- and health reasons, the patients could not be prevented from intake of food or drink at other times and in between the times of exposure. It can therefore not be precluded that nutrition intake may have affected the results.

A challenge with clinical trials that test commercially available products is that they are often sponsored by the industry. The inherent suspicion that the results may not be completely independent is then difficult to rule out due to the potential economic profits of positive results (158). Our RCT has received no financial support other than funds from the University of Bergen.

5.2 Ethical considerations

In this thesis, two of the studies, the in vitro study and the RCT, required approval by the Regional Ethical Committee (REK). The in vitro study required approval for including participants as donors for the oral mucosa model, and for storing the cells according to the approval and the guidelines of the University of Bergen. The RCT obviously required REC approval for including vulnerable patients for the interventions. Ethical issues are central to palliative research. The Helsinki Declaration acts as a form of ethical constitution for human research (152, 159).

Until palliative medicine was established as a speciality, knowledge within the field was built on tradition and experience (20). Allowing research on groups and individuals who are entitled to special protection is necessary because lack of relevant knowledge may lead to inappropriate treatment and put patients at risk. Research is

thus a necessity, also for demented, elderly, children, pregnant and dying. At the same time, strict limits have been set for research on minors and others who cannot themselves give consent. Such research can only be carried out when it is expected that the research will benefit these groups and if the research cannot be done on other groups (152). The RCT was approved because none of the products had any known side effects, palliative patients can deviate from other patients with similar oral dryness problems and because the participants could benefit from the interventions.

5.3 Findings

5.3.1 Procedures and knowledge of oral palliative care

Daily tooth brushing is for most people essential for perceived well-being. Therefore, it is strange that such care is not equally obvious when taking care of others. Article I showed that 25% of the included institutions did not have procedures for oral palliative care. Presumably, some oral palliative care procedures were undertaken, despite the lack of formal ones. However, a more likely scenario was that the patients received no oral care although procedures existed. The latter alternative was indicated in several answers to the open-ended questions in the survey. Explanations were lack of time, insecurity regarding clinical procedures, the feeling of intruding on the patient's privacy or refusal by the patient. The same kinds of responses have been reported in previous studies (160-164). A recent study states that even though oral care is recognized as an essential aspect of nursing, it is often not considered a priority, especially when various complex patient needs have to be managed (165). A Swedish study, which aimed at exploring and describing attitudes relating to xerostomia among health care professionals, found that the condition was considered to be an underestimated and ignored problem, although commonly occurring (166).

Educational issues

The above raises the question of whether the nursing educational programs are adequate concerning oral health. Several studies point to too few teaching hours and insufficient clinical practice in oral health procedures (160-163). Lack of oral-health-

related content in the curricula of nursing education is mentioned as a reason for the refusal to give oral health related care. In a survey study among 235 nurses in the USA, 75% received less than 3 hours of oral health related education/training and 60% reported having no requirements for clinical training in the assessment of oral conditions (164). In a Swedish study among 137 nurses, only four reported having received adequate training in oral care during their education (161). In the same sample, 45% objected to examining the oral cavity and stated patient integrity as the main reason. In contrast, the findings in a Norwegian study from 2009 appeared to contradict that the basic education in oral care of long-time-care (LTC) professionals was inadequate (167). There might therefore be other explanations for the poor oral hygiene in many institutions.

Little prestige and low priority

In our questionnaire study, almost half of the nursing homes did not recognise that oral problems were that important; probably because the personnel were not sufficiently aware of their significance. The results of this study do not indicate whether or not patients from such institutions actually received adequate oral hygiene, but no doubt oral problems did exist there. This also agrees with the results of numerous previous studies, (2, 6, 168, 169).

A positive attitude and sufficient knowledge in healthcare professionals are prerequisites for adequate care of the oral cavity in serious illness. Nevertheless, studies indicate that it is difficult to encourage healthcare providers to be proactive with oral disease prevention and to promote good oral care (160, 162, 163). Researchers have found a disparity between recorded and true prevalence of xerostomia (170). The reason is unclear, but probably it reflects both healthcare professional-related factors (e.g. perception that the symptom is unimportant) and patient-related factors (e.g. perception that other symptoms are more important) (64, 170, 171).

Obviously, fields that are perceived as more challenging are more popular and prestigious (172). Geriatrics and psychiatry are among the lowest ranked specialities

(173). It can be assumed that similar rankings regarding daily work tasks exist in nursing and that these will influence priorities in care. Miller suggests that the trend toward inadequate oral care is caused by a reluctance to develop best practice guidelines and nursing protocols, relegating the responsibility for oral care to lower grades of nursing staff, which amplifies the negative effect (122). In a survey among physicians and medical students, low prestige scores were given to diseases and specialities associated with chronic conditions, with less visible treatment procedures and with elderly patients (172). It can be assumed that the perception of oral palliative procedures by health personnel is similarly assessed.

Does the dental team have a role in palliative medicine?

During the implementation of the RCT study, some nurses at Haraldsplass hospital expressed that the patients might be more interested in oral care if offered by a dentist or hygienist, than by a nurse. This was not investigated in our study, but raises an important question: Should oral care in institutions be carried out by dental professionals rather than by the nursing staff? Wiseman claims that the importance of dental care is often overlooked because the dentist is not included in the palliative care team (8). In a Japanese study it is claimed that more dental services should be made available (174).

In the literature on palliative treatment, cooperation is often referred to, but the dental team is rarely mentioned in that context (175, 176). The fact that the category dry mouth, which was added to the Norwegian version of the ESAS form in 1999, was removed in the revised version from 2010, expresses a lack of focus on and understanding of oral issues, rather than oral problems not being of significant importance (47). The discipline palliative medicine is relatively new and is constantly evolving. Being aware of and positive to cooperation with other disciplines opens the possibility of focus on oral problems. However, it is a well-known challenge that oral health is not equated with general health (177-179) even though oral health is closely related to systemic health (180).

Expectations of patients and relatives

In regard to the attitudes of the healthcare professional on the one hand and the expectations of the patients on the other: Patients participating in the RCT indicated that they did not want to bother the nursing staff unnecessarily. Lack of expectations about oral care may not only be in the nursing staff at the hospital, but also in patients and relatives.

Differences between nursing homes and hospitals

One reasons why far more hospitals than nursing homes stated that they considered oral problem of significance, could be that the type of patients and treatments differs significantly between hospitals and nursing homes. Previous studies indicate that oral care is more emphasized in intensive care units (181) than in palliative care units (169), presumably because it is necessary to protect patients from aspiration of pathogenic bacteria (182, 183). Hospital departments have more physicians and nurses, whereas assistant nurses with lower education dominate in nursing homes. Differences in occupational groups can also explain the differences in self-perceived knowledge about oral care between nursing homes and hospitals in our questionnaire study.

5.3.2 Glycerol, indication or contraindication for use

Recommendations regarding glycerol as an oral moisturizer in palliative care are remarkably contradictory. Some studies claim that glycerol used for this purpose, is directly unfavourable because of its desiccating effect (122, 136, 138, 184). The national guideline in Norway (139), recommends the use of glycerol as moisturizer in oral palliative care. Despite this being the subject of debate for over 80 years, the guideline is in fact solely based on expert clinical experience, not on good quality scientific evidence. Consequently, it may be questioned if it satisfies the requirements of Evidence-based medicine (EBM) or Evidence-based health care (EBHC), which are defined as “the conscientious, explicit, judicious and reasonable use of modern, best evidence in making decisions about the care of individual patients” (185).

However, in the absence of good scientific evidence, experience based knowledge, such as the Norwegian national guideline, must be used (186).

Biological effect

The conflicting recommendations about the use of glycerol as a moisturizer and knowledge that concentrated glycerol appeared to be dehydrating (187) was a starting point for the desire to study its biological effects in the in vitro study (Study II). Epithelial tissues maintain their homeostasis by balancing the continuous cell loss from desquamation on the surface with proliferation of cells in the basal cell layer (147, 188). Thus, increased proliferation is actually a natural response of a healthy epithelium, as response of increased cell loss(188).

Of concern in this study, however, is the fact that the increase in cell proliferation was overbalancing apoptosis in the two highest concentrations (42.5% and 85%), as indicated by the increase in epithelial thickness. For long-term exposure, one might fear that the use of high concentrations of glycerol would increase the risk of malignant transformation and increase the risk of accumulation of DNA errors with each cellular replication. However, the use of glycerol must be seen in a clinical context and in the time frame of palliative patients. The study shows that glycerol does not affect tissue differentiation or tissue integrity, indirectly suggesting a maintained barrier function. Glycerol's desiccating effect was not investigated in this study.

Clinical short-term effect

In our RCT (Study III), glycerol produced significant reduction in xerostomia just after application, but was no longer efficient after 2 hours. An American study from 1997 indicates the opposite tendency; that patients with xerostomia became drier the first few days after lubrication, regardless of the humectant received, but experienced improvement from their dry mouth after four days (189). A limitation with our study was that the follow-up time after intervention was only 2 hours and the fact that the products were only applied once, thus precluding the possibility of investigating the long-term effect. Nevertheless, in this patient group, the immediate effect is more

important. A weakness with several studies referring to the use of glycerol is that the concentration of glycerol is not reported (123, 189), despite the fact that the concentration is crucial in regard to its hygroscopic effect (190). In this study, we offer scientific evidence that while glycerol gives immediate relief and probably is not harmful to the tissue by short-term use and at low concentrations; its moisturizing effect disappears quickly.

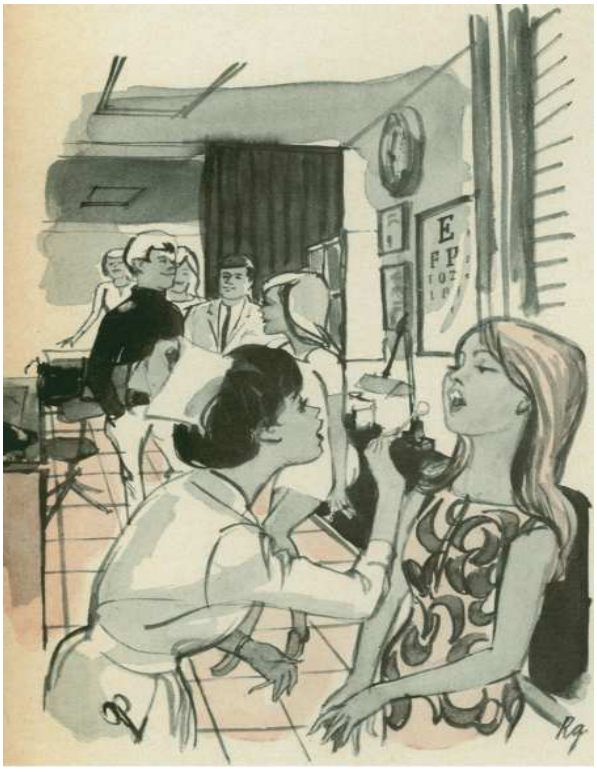

Fig. 7. *An old illustration of the use of glycerol against dry mouth reflects an 80-year-old problem that still remains unresolved. Showing a group of college students testing different combinations of lemon juice and glycerol for oral care in 1969.*
Source: The American Journal of Nursing (1969)

5.3.3 Clinical findings in the RCT

Palliative care patients may experience that several bothersome symptoms and conditions may occur because of oral dryness and several studies highlight clinical findings and symptom management in palliative care (3, 91, 165, 169, 191). Several studies address oral symptoms resulting from cancer or radiotherapy, such as pain, soreness, swallowing problems, speech impairment, oral infections, increased incidence of caries and others (3, 122, 165, 169, 192). The symptoms are consistent with the patient group in this RCT. Most patients in the study used opioid analgesics, sedatives, neuroleptics and anticholinergic drugs; some also used neuropathic pain medication, octreotide against bowel obstruction and proton pump inhibitors against reflux ailments. Many intervention studies have been conducted with regards to the management of dry mouth (10, 193) on a wide range of different patients with diverse complaints caused by autoimmune conditions, hormonal disorders, immune disorders, patients undergoing haemodialysis, medication-induced xerostomia, and radiotherapy (10, 193). In the Cochrane rapport from 2011 on topical therapy interventions for the management of dry mouth, randomized crossover trials were considered to be an appropriate design for research in this area. Dry mouth symptoms are likely to be stable over time and topical therapies are likely to offer relatively short-lived effects, which are likely to be reversible (10). A randomized crossover design was applied in this RCT.

Palliative patients as participants

The reason why palliative patients were chosen for our RCT was twofold: It was assumed that their need for oral care might be greater and/or different from less compromised patients, and that in such patients, possible differences between products might be more discernible. To our knowledge, very few clinical trials have compared the effect of saliva substitutes in palliative patients. Only one clinical trial on palliative patients was found (194). Sweeney et al. tested two oral sprays, a mucin-containing spray versus placebo, in 35 hospice patients. Unlike our RCT, Sweeney had a follow-up period of 14 days, where the patients used the allocated oral spray as much as possible every day, and 26 patients remained in the study on day 14. There

was no statistically significant difference between those on active and placebo when oral symptoms were recorded. The investigators still concluded that both sprays provided some degree of symptomatic relief of oral dryness for many of the participants, as they wanted to keep the spray after the intervention period.

What possible alternatives could there be?

In our RCT we found effect after two hours for two of the products. Since a majority of patients tolerated these products poorly, it may be natural to think of alternatives. An obvious alternative is the possibility of modifying the two products that had effect; if possible to dilute Salient® to make it less sticky or add another flavour to Aequasyal®. Glycerol gave good relief immediately, but quickly lost its effect. One can question whether there is in fact any significant difference between such a low concentration as 17% glycerol and pure water.

A major problem, which will not be solved regardless by application of topical saliva substitutes in the mouth, is the dryness that extends down the throat. This was often the largest problem for the most severely affected and ill patients. Pilocarpine, which is a parasympathomimetic drug, is, to our knowledge, rarely used in Norwegian hospitals and nursing homes. In some studies, unpleasant dose-dependent side effects have been shown, while several studies show that pilocarpine is more effective than placebo (195). In patients with functioning salivary tissue, Pilocarpine may offer an opportunity that will also help to moisturize the pharynx. But experience and evidence for the application in palliative patients is sparse.

Intravenous (IV) fluid supply is used and there has recently been a slight shift from the perception that terminating IV fluid completely in the terminal phase is correct, to instead making individual adjustments according to patients' and relatives' wishes, since there is contradictory evidence and experience (196-198).

Oral care is valued

As there is no optimal oral moisturizer available, it is nevertheless important that oral care is carried out. Of the patients in the RCT, almost all expressed that having teeth and gums cleaned, provided relief and some commented that the cleaning was even

the best part of the intervention procedure. A majority of the patients in the RCT were unable to carry out daily dental- and oral care themselves (Fig. 8 and 9).

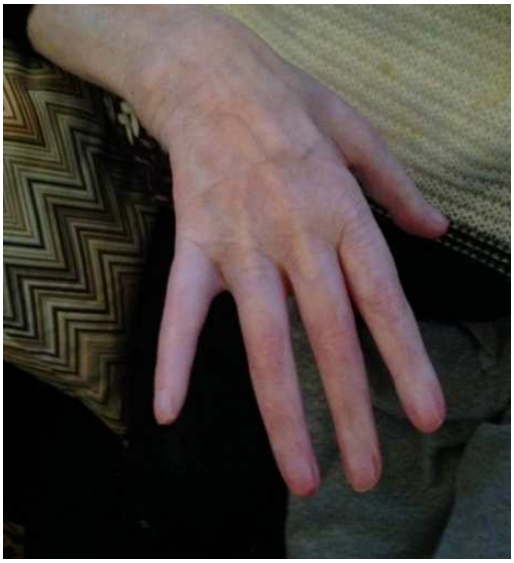

Fig. 8. *One of the patients in the RCT study had syringomyelia as an additional diagnosis. The disease had led to stiffness in the arms and fingers so that she could not open a toothpaste tube. She had not been able to brush her teeth for several weeks.*
Photo: S. Kvalheim (with permission from the patient)

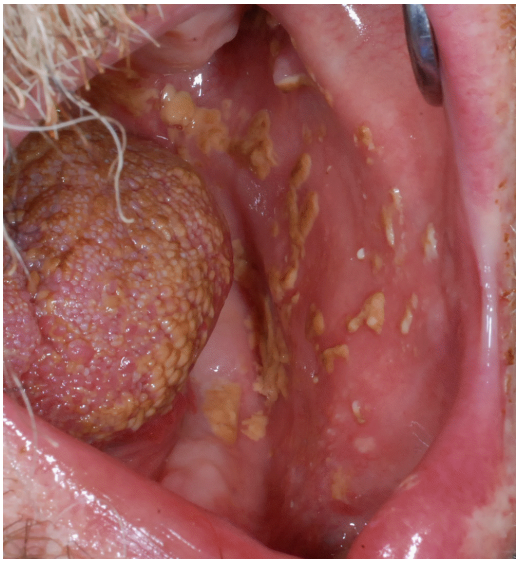

Fig. 9. *This man from the RCT study wanted help because he felt he had an "outgrowth" in his throat. The discomfort was due to large amounts of fungi.*
Photo: S. Kvalheim (with permission from the patient)

6. Conclusions

6.1 Overall conclusion

Oral palliative care has only to a small degree been studied. Research within this field must be considered pioneering work. For that reason, this thesis was initiated with the survey study that indicated how this treatment was implemented in practice by the relevant institutions, and what attitudes existed among care givers towards such treatment. The widely differing products used suggested a lack of solid scientific basis and uncertainty regarding their use. An experimental, biological laboratory study was therefore initiated to explore the biologic consequences on a cellular level of the use of different concentrations of glycerol, the most commonly used product in Norwegian health institutions. Finally, an RCT-study was performed in order to investigate how the most recommended products were received by those most needy of oral care: palliative patients. The hope and aspiration have been that this thesis might make some contribution towards widening the body of knowledge within the field of oral palliative care.

6.2 Specific conclusions

Xerostomia is a major problem among palliative care patients. Nevertheless, many nursing homes and hospitals lack proper procedures for oral palliative care. There are obvious challenges related to lack of knowledge and attitudes in relation to such care.

Glycerol, tested on an oral mucosa model, does not seem to harm the mucosa on a cell-level, provided it is applied in a low concentration and used for a limited period of time. Higher concentrations (42,5 % and above) may result in increased proliferation and cell-apoptosis, which can theoretically be associated with malignant transformation of the epithelium.

Among the agents tested in this RCT study, glycerol was the preferred product despite the fact that it was the least efficient 2 hours after application, at which time it had lost its effect. Both of the other two products, Aequasyal® and Salient®, gave

statistically significant improvement in xerostomia, but were not well tolerated by this group of patients due to their taste or consistency.

The study hypothesis of no difference between the effects of the three products is rejected.

7. Future Perspectives

Scientific approach

Several aspects of dry mouth in palliative care patients need to be further investigated:

- Mucosal and salivary properties in seriously ill patients with xerostomia need to be investigated, in order to develop well adapted products for appropriate oral care
- Other kind of products
- Other approaches for hydration

Clinical and educational approach

Oral health problems in severely ill patients should be made known to healthcare professionals in hospitals and nursing homes. Emphasis must be placed on information about the relationship between oral and general health, as well as on future models for health care education and organization of oral care in institutions.

Patient-centered approach

It seems self-evident that oral health should be implemented consistent with other kinds of health care.

- Oral care must be recognised as a necessary daily care and the nursing staff must be responsible for ensuring that oral care procedures are carried out.
- It must be assured that lack of awareness of oral palliative care in healthcare professionals does not prevent patients from accessing symptomatic treatment.
- Patients should be given the opportunity of trying different lubrication products or alternative ways of hydration.

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Papers I, II and III

Paper I

End-of-life palliative oral care in Norwegian health institutions. An exploratory study.

Kvalheim SF, Strand GV, Husebø BS, Marthinussen MC.

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I

Gerodontology

Original article

End-of-life palliative oral care in Norwegian health institutions. An exploratory study

Siri F. Kvalheim[1], **Gunhild V. Strand**[1], **Bettina S. Husebø**[2,3] and **Mihaela C. Marthinussen**[1]

[1]Department of Clinical Dentistry, University of Bergen, Bergen, Norway; [2]Department of Global Public Health and Primary Care, Center for Elderly-and Nursing Home Medicine, University of Bergen, Bergen, Norway; [3]Center for Age-Related Medicine, Stavanger University Hospital, Stavanger, Norway

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End-of-life palliative oral care in Norwegian health institutions. An exploratory study

Objective: To explore circumstances surrounding procedures and knowledge regarding oral care for terminal patients in Norwegian healthcare institutions.
Methods: A questionnaire was distributed to randomly selected hospitals (n = 19) and nursing homes (n = 57) in central and rural parts of Norway. The questionnaire included three closed-ended and three open-ended questions about oral care for terminal patients. If procedures existed, the respondents were asked to enclose or describe them.
Results: The response rate was 84% for hospitals and 79% for nursing homes. Of the responding institutions, 25% had no oral care procedures, nor did 48% recognise their importance. Insufficient knowledge about oral care was reported by 39%. Twenty-one different procedures were identified, and a great number of oral care products used. The most common was glycerol, used by 36% of the institutions. Only 2% used a concentration below 30% – the limit above which the glycerol has a desiccating rather than a moistening effect. The most common patient complaint was dry mouth (49%), followed by plaque, food particles and fungus infections, each experienced by 19%. The most common problem for the personnel was lack of knowledge (43%) and patient cooperation (38%).
Conclusions: Some terminal patients do not receive adequate palliative oral care in Norwegian healthcare institutions. Those that do are exposed to a great number of undocumented procedures and sometimes harmful products. There is a need for evidence-based procedures for oral care for terminally ill patients in health institutions, establishing interprofessional palliative healthcare teams and in particular improved training of the nursing staff.

Keywords: palliative care, oral health, oral hygiene.

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Introduction

Ideally, dying should be painless, peaceful and dignified. Palliative care for dying patients is defined as comprehensive, interdisciplinary care of patients and families facing a terminal illness, focusing primarily on comfort and support[1]. The more specific concept of palliative oral care has been defined as the management of patients with far-advanced disease where the oral cavity has been compromised, either directly by the disease or indirectly as a consequence of its treatment[1]. The term 'general palliative care' is often associated with cancer treatment, but WHO suggests that expertise from this field should be extended to treatment of other groups of severely ill persons such as elderly nursing home residents[2].

The majority of terminally ill patients have a wide range of oral symptoms, including speech difficulties. The oral cavity is frequently dry; the tongue adheres to the mucosa and impedes the expression of remarks, gratitude or desires. Increased salivary viscosity may inhibit swallowing and taste[3,4]. Thirst is a common problem for severely ill patients. Dehydration may be due to reduced liquid intake, diarrhoea, fever or vomiting. Dry lips can lead to cracking, particularly when smiling, and oral infections and pain occur easily when the lubricating, protective and antimicrobial effect of saliva is lost[5,6]. An important

aspect of palliative care is to prevent halitosis, which can be off-putting for relatives and friends who wish to bid farewell. This is a time when dignity and self-respect are essential[7].

Norwegian data indicate that 56% of terminal patients complained about dry mouth[8]. In addition, 49% complained about thirst and 74% about altered eating habits, which may to some extent be related to xerostomia.

Such problems are probably mainly caused by the medications used in palliative medicine (for instance opiates, glucocorticoids and antidepressants) in combination with mouth breathing and dehydration. Infusions may to some extent alleviate the dryness of the mouth and thirst. However, these are only used when likely to improve the patient's quality of life; otherwise, oral care with frequent moistening of the oral mucosa should be implemented[9,10].

The object of all kinds of palliative care is to provide patients with relief from the symptoms, pain and stress of a serious illness – whatever the diagnosis and to improve quality of life for both patient and family.

To this end, WHO published in 2004 palliative care guidelines[11]. However, their recommendations on oral care are fairly general, and no evidence for their efficacy is provided. They do not specify guidelines to alleviate specific symptoms related to dry mouths and lips, nor the optimal frequency of such treatment.

Searching in the literature for evidence-based procedures reveals that little research has been published within this field. Still, many different procedures for oral care for critically ill patients exist[12–15]. However, with a few exceptions like the documented effect of usage of electric toothbrushes and fluorides on oral health, they are based on expert opinion[15]. Sometimes recommendations rely on research made on healthier target populations[16]. Some authors claim that oral care is performed every 2–4 h or on a daily basis[17]; others report that such procedures are not always carried out in hospitals and nursing institutions caring for these patients[13].

Palliative oral care is shown to be effective in alleviating the oral symptoms of terminally ill patients[18]. However, for a number of reasons, oral care is not always adequate[19]. The time and resources allocated to this task may be insufficient, and the staff were inadequately trained and therefore lack the necessary skills. Another important reason is that the recommendations in many respects are not evidence based. Undocumented use of glycerol and undefined frequency of oral care are examples of this. In fact, it has been claimed that products commonly used may be contraindicated for routine oral hygiene, such as hydrogen peroxide, sodium bicarbonate and lemon and glycerol[20,21].

Like in the above-mentioned studies, no formal, generally accepted and evidence-based Norwegian procedures on oral care for end-of-life patients exist; local and differing ones have been developed and applied with little or no evidence base. No legislation compels all health institutions with terminal patients to implement systematic palliative care, although such plans are being discussed. However, all patients in Norwegian health institutions have a legislated right to 'adequate and individually adapted treatment' (including oral care). What this means in practice is presently up to the caregivers to decide, if possible in cooperation with the patient. Some regional hospital and hospices have palliative units – most do not.

To provide end-of-life patients with adequate evidence-based oral care, an important first step is to explore the current situation with a fairly open overview. With this in mind, the purpose of the present investigation was thus (i) to record to what extent Norwegian institutions have procedures for oral care for terminal patients, (ii) to study the content of these, (iii) to record the level of knowledge of the nursing staff regarding palliative oral care and (iv) to identify possible problems associated with oral care. The intention of this exploratory study is to reveal areas that may later be the subject of targeted experimental research in an effort to establish evidence-based procedures.

Methodology

A questionnaire regarding palliative oral care for end-of-life patients was developed by the authors all of whom have extended experience within this field. The items were the result of a consensus within the group. A pilot study was conducted prior to this investigation among a small group of nurses to ascertain that the wording was comprehensive and easily understood. The wording was adjusted according to received responses. The questionnaire was sent by mail to 76 Norwegian health institutions. In the letter, complete contact information was given, including main line telephone number, cell phone number and e-mail address. Self-addressed and stamped envelopes were provided. The health institutions were randomly chosen by drawing lots, and one hospital and three nursing homes for each of the country's

19 counties were thus selected. The charge nurse or the deputy was asked to complete the questionnaire.

An accompanying letter explained that the study concerned oral treatment of patients who matched the Liverpool Care Pathway (LCP) criteria[22]: the patient should have been considered, by an interdisciplinary team, to be dying. The life expectancy is then usually a matter of days or even hours. This often implies that the person is bedridden, incommunicative for extended periods, can drink only small quantities of liquid and cannot swallow tablets. After 6 weeks, a reminder letter was sent to all institutions.

The questionnaire contained three items with predetermined response categories (wording and response categories shown in Table 1). It additionally contained three open-ended items, which were designed to explore further the circumstances surrounding palliative oral care. The open-ended approach was chosen to explore, without the limitation of predetermined categories, the thinking behind the procedures. In the first one of these, the respondents were asked to describe or document what procedures for palliative oral care for end-of -life patients existed; in the second one, to report who had formulated the procedures; and in the third, to explain what characterised oral problems for the terminally ill patients and for the personnel treating them. The responses to the open-ended items were later interpreted and categorised by the first author, who, when in doubt, conferred with the other authors.

During this categorisation, procedures with approximately similar methodologies and use of oral care products were identified. An effort was made to assess each procedure as objectively as possible. Within each identified procedure, the methods and/or oral care products used were similar, if not necessarily completely identical. Who authored the procedures and what characterised oral problems for the terminally ill patients and for the personnel treating them were similarly recorded.

Table 1 Items with predetermined categories. Distribution of responses and comparisons between health institutions. Percentages in brackets.

Item	*Hospitals n (%)*	*Nursing homes n (%)*	*Overall n (%)*	*p-value*
Do you have procedures for oral care for the dying patient?				
Yes	13 (81)	33 (73)	46 (75)	0.74[a]
No	3 (19)	11 (24)	14 (23)	
Do not know	0 (0)	1 (2)	1 (2)	
Do you feel that you have sufficient knowledge of this type of oral care?				
Yes	11 (69)	26 (58)	37 (61)	1.00[a]
No	5 (31)	14 (31)	19 (31)	
Do not know	0 (0)	5 (11)	5 (8)	
Do you recognise that oral problems are of important significance?				
Yes	12 (75)	20 (44)	32 (52)	0.07[a]
No	3 (19)	20 (44)	23 (38)	
Do not know	1 (6)	5 (11)	6 (10)	

[a]In the comparisons, 'Do not know' and 'No information' are not included.

Statistical methods

The frequencies of the responses were counted. Groups were cross-tabulated in 2×2 tables and compared using Fisher's exact test. In comparisons between the groups, the variables were dichotomised as follows: regarding all the variables in Table 1, the responses 'Do not know' were excluded from the analyses. Regarding mouth cleaning procedures shown in Table 2, the responses 'Teeth only', 'Mucosa only' and 'Teeth and mucosa' were combined into one category (defined as 'Cleaning procedures'), vs. 'Do not clean'. Similarly, the responses 'Glycerol ≤30%', 'Glycerol >30%', 'Glycerol, unspecified concentration' and 'Other than glycerol' were combined (defined as 'Use lubricator') vs. a 'No lubrication'.

The analyses were performed by means of SPSS 20 (IBM Corp, IBM Statistics for Windows, Version 20.0, Armonk, NY). A 5% significance level was chosen for all analyses.

Results

Response rate

The response rate was 16 of the 19 hospitals (84%) and 45 of the 57 nursing homes (79%). The overall response rate was 80%. There are 46 hospitals and 1050 nursing homes in Norway. The responding institutions thus represented 16/46 (35%) of all hospitals and 45/1050 (4%) of all nursing homes in the country. No responders had questions relating to the questionnaire.

Items with predetermined categories

The frequencies of the items with predetermined categories, overall and split into hospitals and

Table 2 Open-ended items. Distribution of frequently occurring actions used within the procedures and comparisons between health institutions. Percentages in brackets.

Element	*Hospitals n (%)*	*Nursing homes n (%)*	*Overall n (%)*	*p-value*
Assessment of oral condition				
Yes	4 (25)	0 (0)	4 (7)	0.004
No information	12 (75)	45 (100)	57 (93)	
Lip moistening				
Yes	11 (69)	24 (53)	35 (57)	0.38
Do not moisten/no information	5 (31)	21 (47)	26 (43)	
Mouth cleaning procedures				
Teeth only	1 (6)	1 (2)	2 (3)	1.00[a]
Mucosa only	3 (19)	12 (27)	15 (25)	
Teeth and mucosa	8 (50)	19 (42)	27 (44)	
Do not clean/no information	4 (25)	13 (29)	17 (28)	
Cleaning dentures				
Yes	5 (31)	15 (33)	20 (33)	1.00
No information	11 (69)	30 (67)	41 (67)	
Use of lubricator				
Glycerol ≤ 30%	0 (0)	1 (2)	1 (2)	0.57[b]
Glycerol > 30%	1 (6)	5 (11)	6 (10)	
Glycerol unspecified	1 (6)	14 (31)	15 (24)	
Other than glycerol	5 (31)	4 (9)	9 (15)	
Do not use lubricator	9 (56)	21 (37)	30 (49)	

[a]In the analysis, the variables 'Teeth only', 'Mucosa only' and 'Teeth and mucosa' are combined into one category defined as 'Cleaning procedure'.
[b]In the analysis, all glycerol concentrations and lubricator other than glycerol are combined into one category defined as 'Use lubricator'.

nursing homes, are shown in Table 1. Of the 61 participating institutions, 15 (25%) had no procedures, 24 (39%) reported insufficient knowledge of this type of oral care, and 32 (52%) still recognised that oral problems were of important significance.

Open-ended items

Procedures and implementation. Eleven of the 13 hospitals (85%) and 18 of the 33 nursing homes (55%) that did have procedures for palliative oral care for end-of-life patients returned printed copies of these. In addition, one of the 13 hospitals (8%) and 14 of the 33 nursing homes (42%) submitted exhaustive descriptions of the procedures. One hospital and one nursing home (8% and 3%, respectively) claimed to have procedures, but did not document them.

Categorisation of the responses revealed 21 different identifiable procedures. These differed widely, both in terms of what actions were implemented and oral care products used. Within the 21 procedures, five actions were identified (Table 2).

The oral condition was assessed by four of the 61 institutions (7%). If so, the oral condition was assessed at arrival and daily written records were made of conditions and interventions. Lip moistening was used by 35 of the institutions (57%).

While 44 institutions (72%) either cleaned the teeth, mucosa or both, 17 (28%) did neither. The teeth were cleaned with soft toothbrushes in 16 of the 21 (76%) identifiable procedures. Most institutions used sterile sponges on locking tweezers or disposable oral swabs for cleaning the mucosa.

Even though all procedures recommended that the patients' dentures were cleaned, only 20 (33%) actually did so.

In 22 of the institutions (36%), various concentrations of glycerol were used to lubricate the mucosa, but only one institution (2%) reported using a concentration below 30% (Table 2). However, three respondents pointed out that glycerol was not used because of the danger of drying out the mucous membranes. In 30 institutions (49%), no lubricator was used.

A wide variety of oral care products was used in the various procedures (Table 3). Thus, seven

Table 3 Products used in oral care procedures.

Lubricating lips	*Cleaning teeth*	*Cleaning mucosa*	*Lubricating mucosa*
Eucerin liniment®	SLS-free toothpaste	H_2O_2[a] and water 1:3	Glycerol[b]
Glycerol[a,b]	Toothpastes[a]	H_2O_2 3%	Glycerol solution 17%
Vaseline[a]	NaCl 9 mg/ml	Chlorhexidine, undiluted	Glycerol solution 50%
Blisex®		Chlorhexidine and water 1:2	Glycerol solution 70%
Lypsyl®		Chlorhexidine and water 1:3	Glycerol with peppermint oil
Lip stick[a]		Vademecum®	Glycerol and Chlorhexidine[b]
Lip cream[a]		NaCl 9 mg/ml	Xylocaine/Lidocaine viscous®
		Tap water	Xylocaine/Lidocaine viscous®,
		Selters[c]	Paracetamol mixture® and cream
		Chamomile or sage tea	Panodil mixture® and cream 1:1
		'Düsseldorf mixture'[d]	Pure cream[a]
		Triclosan[a,b]	Zendium saliva®
			Zendium gel®
			Groundnut oil[a]
			Saliva gel[a]
			Oralbalance®
			Mouth moisturiser®

[a]Unspecified.
[b]Concentration unspecified.
[c]Carbonated water containing salts and minerals.
[d]Sterile water with baking soda, mycostatin and chlorhexidine.

different products or combinations of products were used for lubricating lips, three for cleaning teeth, 12 for cleaning mucosa and 16 for lubricating mucosa. In addition to the widespread use of glycerol mentioned above, various concentrations of chlorhexidine were frequently used. Even paracetamol was used in various mixtures with xylocaine and/or cream.

Authorship of the procedures. In 11 of the 13 hospitals (85%), the procedures had been authored by nurses and in two (15%) by physicians. Similarly, the procedures were authored by nurses in 16 of the 33 nursing homes (48%) and in one case (3%) by a physician. The nursing homes differed from the hospitals in that the procedures had also been authored by the public dental service in 10 of them (30%), copied from textbooks in four (12%) and written by others in two (6%).

Oral problems for patients and treating personnel. In regard to the question of problems experienced by the patient, three of the 16 hospitals (19%) and 21 of the 45 nursing homes (47%) failed to respond. Of the responding 37 institutions, some mentioned more than one problem. Accordingly, the percentages exceed 100 in sum. A total of 67 responses were recorded.

The most frequent problem was dry mouth, which was reported by 17 of the 37 responding institutions (46%), and with descending frequency plaque, food particles and fungus infections, each reported by 7 (19%); sores and scab, each reported by 6 (16%); viscous ropy saliva and chapped lips, each reported by 5 (14%); reduced appetite and pain, each reported by 4 (11%); and dysphagia, halitosis, coughing and problems using dentures, each reported by 1 (3%).

The problems experienced by the personnel were lack of knowledge/experience/routine, which was experienced by 16 of the 37 responding institutions (43%), lack of patient cooperation by 14 (38%), that oral problems were not prioritised by 8 (22%), difficult access to the mouth by 4 (11%), lack of resources by 3 (8%) and retching by one (3%).

Comparisons

There were no significant statistical differences between hospitals and nursing institutions for any of the variables in Table 1. The only difference between the institutions was whether or not the oral condition of the terminal patients was assessed on arrival (Table 2). Whereas such an assessment was made by four of the 16 hospitals (25%), none of the nursing institutions did so, showing a highly significant difference ($p = 0.004$).

A comparison between those institutions that did have procedures for end-of-life palliative oral care and those that did not showed no significant difference in reported level of knowledge ($p = 0.104$) nor in recognising that the oral condition represents a significant problem ($p = 0.350$).

Discussion

In this investigation, an attempt was made to assure that the results could be regarded as representative for the way palliative oral care for end-of-life patients is implemented in Norwegian health institutions. The method of selection assured geographical distribution throughout the country, in that all counties were represented. Moreover, the institutions within each county were randomly selected. The relatively high number of institutions selected (76), the high response rate (84% for the hospitals and 79% for the nursing homes) and the fact that the procedures to a large extent were dispatched as written procedures also testify to the validity of the results. The fact that no responder felt the need for further clarification points in the same direction.

However, the fact that only the charge nurse or deputy completed the questionnaire might be considered a limitation, primarily because there is no guarantee that the reported procedures were actually implemented in the wards. A charge nurse has mainly administrative responsibilities and does not normally inspect or participate in patient care. Consequently, the results of this investigation only reflect what the charge nurses or deputies report. On the other hand, the head nurse of responding institutions that had current procedures regarding palliative oral care for end-of-life patients would no doubt report them.

Regardless, the above limitation does not detract from the most surprising and disturbing finding that one of four institutions had no procedures at all in this respect. However, it cannot be precluded that this result may in part be related to the fact that interprofessional teams with specific responsibility for palliative care are only occasionally established – typically in the larger regional hospitals and in some hospices.

The procedures varied widely, both in terms of choice of method, and especially in selection of oral care products. It may be considered remarkable that as many as 21 different methods for palliative oral care were identified. Also, the use of oral care products (Table 3) was sometimes contradictory. As an example, glycerol solution was used by 36% of the institutions for lubricating mucosa. It is a matter of concern that 10% of the institutions used glycerol at higher concentrations than 30% (Table 2). If exceeding this concentration the product becomes hypertonic, its use will have a desiccating effect[19,23]. This is contrary to a major purpose of oral palliative care, which is to prevent dehydration of the mucous membranes[24], and is especially important for patients with dry mouths and mouth breathers, conditions common in dying patients.

Also questionable was the use of chlorhexidine for cleaning mucosa. It has been argued that this product is well suited because of its antimicrobial properties, which might reduce the risk of aspiration pneumonia caused by oral bacteria[25]. On the other hand, it has also been argued that chlorhexidine should not be used because it can cause burning and drying of the mucous membranes and may have an adverse effect on the normal oral flora[26].

In several of the procedures, a solution of hydrogen peroxide was used (Table 3), probably because of its expectorant effect[27]. In some procedures, the concentration was unspecified; in others, a 3% solution was used. This is a considerably stronger solution than the 0.5% concentration recommended in the Oxford Textbook of Palliative Medicine[28]. Even more peculiar was the use of mixtures containing paracetamol (acetaminophen) used for lubricating mucosa. True, paracetamol is used for the relief of pain, however, only if administered gastrointestinally; no effect can be expected when applied on the mucosa.

'Düsseldorf mixture' contains an aqueous solution of baking soda, mycostatin and chlorhexidine. The mixing ratios are, however, such, that it is highly uncertain whether this has any effect.

In addition to these products, the wide variety of other substances specified in Table 3 indicates much uncertainty among Norwegian health institutions in regard to palliative oral care for end-of-life patients. This is corroborated by the present findings that 37% of them stated that they had insufficient knowledge of the subject and that 25% had no procedures at all. To some extent, this uncertainty may be explained by the fact that there is little evidence in the literature that one oral care agent is better than another[20]. Similar uncertainty has been observed in other countries[29,30].

It has been shown that significant and consistent improvement of oral health can be obtained using specific protocols[13]. However, the fact that only 52% of the presently studied institutions

recognised that oral problems were of significant importance (Table 1) and that 22% reported that oral problems were not prioritised indicates that palliative oral care is not highly appreciated in Norway. That none of the nursing homes assessed the oral condition as opposed to 25% of the hospitals, although statistically significant, should probably not be attributed much importance. Most likely, when patients are admitted to a nursing home, they would already be assessed by a hospital.

One might perhaps expect that institutions that had procedures for palliative oral care were more knowledgeable than those that did not. Confusingly, this hypothesis was not corroborated in the present study, neither in terms of their reported level of knowledge nor their recognition of the oral condition as a significant problem. These findings are all the more surprising in view of the fact that death in Norway has become largely institutionalised and specific, and/or individualised procedures related to all terminal diseases have been developed with the intention of reducing the physical and mental strain that terminal illnesses entail.

The confusion regarding palliative oral care exhibited by Norwegian health institutions is, however, not a local phenomenon. The same degree of confusion appears to be present in other European countries such as the United Kingdom (A. Davies, personal communication), Sweden[31] and Holland[32] and is probably mainly caused by a lack of evidence-based effective procedures, which are indeed conspicuously absent in the literature[33,34]. In fact, to the best knowledge of the authors, experimental studies pertaining to the efficacy of palliative oral care procedures and products are wanting. This demonstrates the urgent need for research in these matters so that evidence-based national and international procedures can be established.

Conclusion

The results of the present study indicate that the practices regarding palliative oral care for end-of-life patients in Norwegian institutions are both unstructured and haphazard. Some terminal patients do not receive adequate oral care as no procedures to that effect exist. Sufficient funding for establishing interprofessional oral health care teams and improved oral care training of nurses and other front-line caregivers within this field is necessary. The empirical data, on which institutions base their procedures, are for the most part not evidence based, indicating that the scientific basis for specifying methods and oral care products is inadequate. There are obvious methodological problems with research in this area, but these should be overcome to provide good care in a way that comforts the dying.

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Correspondence to:
Siri Flagestad Kvalheim, Department of Clinical Dentistry, University of Bergen, P.O. Box 7800, 5020 Bergen, Norway,
Tel.: +47-55586488
Fax: +47-55586568
E-mail: Siri.Kvalheim@ok.uib.no

Paper II

Effect of glycerol on reconstructed human oral mucosa.

Kvalheim SF, Xenaki V, Kvalheim A, Lie SA, Marthinussen MC, Strand GV, Costea DE.

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II

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Effect of glycerol on reconstructed human oral mucosa

Siri F. Kvalheim[1], Victoria Xenaki[1], Arild Kvalheim[2], Stein-Atle Lie[1], Mihaela C. Marthinussen[1], Gunhild V. Strand[1], Daniela E. Costea[3,4]

[1]Department of Clinical Dentistry, Faculty of Medicine, University of Bergen, Bergen; [2]Oral Surgery Private Referral Practice "Tannteam", Nesttun; [3]Department of Clinical Medicine, Center for Cancer Biomarkers CCBio and Gade Laboratory for Pathology, University of Bergen, Bergen; [4]Department of Pathology, Haukeland University Hospital, Bergen, Norway

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The majority of severely ill patients experience dry mouth. For institutionalized patients, this condition is commonly treated using glycerol as a lubricant. However, because of its possibly desiccating effect, some countries do not advocate the use of glycerol. This study aimed to investigate dose-dependent effects of glycerol on homeostasis and tissue integrity of in vitro-reconstructed normal human buccal mucosa (RNHBM). Primary keratinocytes and fibroblasts were isolated and expanded from biopsies of mucosa from eight healthy volunteers. Ninety-six samples of RNHBM were prepared and exposed for 24 h to 17%, 42.5%, or 85% glycerol, or to distilled H_2O (control). Sections were stained with haematoxylin and eosin (H&E) to evaluate epithelial thickness or used for immunohistochemistry to measure expression of Ki67 (proliferation), cleaved caspase-3 (apoptosis), and E-cadherin (tissue-integrity). Positive cells and cell layers, as detected by immunohistochemistry, were counted. Epithelial thickness, proliferation, and apoptosis were significantly increased by exposure to 42.5% and 85% glycerol. No significant differences in apoptosis or proliferation were found between controls and RNHBM exposed to 17% glycerol. E-cadherin expression was not significantly affected by exposure to any of the concentrations of glycerol tested. This study shows that glycerol affects tissue homeostasis, but not tissue integrity, of RNHBM at glycerol concentrations above 42.5%.

Siri Flagestad Kvalheim, Department of Clinical Dentistry, Faculty of Medicine, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

E-mail: Siri.Kvalheim@uib.no

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A majority of patients in palliative care have problems with dry mouth caused by medication, mental status, or as a direct result of the mortal condition (1, 2). Dry mouth causes a variety of problems that commonly affect the disease negatively and contribute to reduced quality of life in the patient's last stage of life. Lack of saliva in severely ill patients often leads to infections of the mouth and throat, discomfort, pain, dysphagia, speech problems, and loss of appetite (3, 4).

A large number of artificial saliva products are commercially available. In recent years, there has been increasing focus on saliva research, mainly based on products containing nanoparticles and stem cells (5). So far, no one has succeeded in making artificial saliva that mimics the complexity of natural saliva (6, 7).

Glycerol is a simple polyol compound. As a result of its chemical structure, it has a large number of different applications: as a sweetening agent; in cosmetics production; as a constituent of soap, candy, and antifreeze; and in the production of different medications. Glycerol is considered non-toxic and safe. The ability to attract water from its surroundings is the basis for its use as a humectant in moisturizers. The hygroscopic properties of glycerol depend on its dilution in water and on the relative humidity of the surrounding air, which is related to temperature (8).

In Norway and many other countries, glycerol diluted in water in different concentrations, from 17% to the pure form of about 85%, is the most commonly applied oral moisturizer in palliative care patients (9). Being cheap, easily available, and easy to apply, glycerol is used for the purpose of oral mucosa lubrication in many other countries, whereas in some countries, including USA, the Netherlands, Great Britain, and Singapore, glycerol applied as an oral moisturizer is not recommended because of its hygroscopic properties, which may cause desiccation (10). The hygroscopic properties of glycerol are well known. Nevertheless, the literature is sparse when it comes to documenting the effect of glycerol at molecular and cellular levels. Its effects on oral mucosa have not yet been systematically investigated and documentation on its biological effects is currently lacking.

Oral epithelium is a stratified squamous epithelium consisting of cells tightly attached to each other and arranged in a number of distinct cell layers. Like epidermis and the lining of the gastrointestinal tract, its normal structure and function require a balance to be maintained between continuous cell loss at the epithelial surface and cell proliferation at the basal cell layer (homeostasis), as well as competent cell-to-cell adhesion (tissue integrity) (11, 12). Among constituent structural

molecules that assemble in order to ensure adequate epithelial cell-to-cell adhesion, cadherin/catenin-based anchoring junctions organize and tether microfilaments to maintain cell-adhesive properties (13, 14). Both in vitro and in vivo studies have suggested E-cadherin as a key player in maintaining cell-to-cell adhesion and tissue integrity. The absence of E-cadherin leads to permeable tight junctions and to altered epithelial tissue integrity and resistance (15). Blocking E-cadherin in vitro inhibits tight junctions in simple epithelia (16), as does genetic loss of epidermal E-cadherin (17).

The objective of this study was to investigate dose-dependent effects of glycerol on homeostasis (cell proliferation and apoptosis) and tissue integrity (E-cadherin expression) of in vitro-reconstructed normal human buccal mucosa (RNHBM). In vitro-RNHBM was chosen as an experimental model because of its similarity to native normal human buccal mucosa (18, 19). Reconstructed normal human buccal mucosa displays a well-differentiated stratified squamous epithelium expressing various markers of proliferation, differentiation, and apoptosis in a pattern similar to that of native buccal epithelium, mimicking closely its architecture and homeostasis (19). Biopsies of 0.5 cm^2 enable the production (cultivation) of RNHBMs from which it possible to produce a large number of parallel tissue sections. These permit the study of the effects of reagents on tissue replicates derived from the same patient. Using these tissues, we were able to perform a standardized study, avoiding direct involvement of patients or experimental animals. The same kind of RNHBM samples have been used previously to study homeostasis and tissue integrity under the influence of khat (20) and sodium lauryl sulphate (SLS) (21).

Material and methods

Tissue material from human donors

Eight biopsies of normal human mucosa with an approximate size of 0.5 cm^2 were obtained from superfluous buccal mucosa of patients undergoing surgical removal of a third molar (Fig. 1A). The indication for this treatment was partially erupted teeth. Only young healthy adults with no subjective or clinical sign of local inflammation at the time of surgery were included. All patients gave informed consent for use of their tissue samples.

For immunohistochemistry and histomorphometry analyses, only RNHBM samples with a continuous epithelium consisting of at least five cell layers were included. Samples were placed in transport medium (Fig. 1B): Dulbecco's modified Eagle's medium (DMEM; Sigma, St Louis, MO, USA) containing 2% antibiotics-antimycotics (Gibco-BRL, Grand Island, NY, USA). The Regional Committee of Medical Ethics in Research approved the project (REK approval: 2015/1851).

Primary cell isolation

The biopsy was cleaned with PBS (Gibco-BRL) and then washed twice with fresh transport medium. In order to separate epithelium from connective tissue, the tissues were transferred to dispase solution [20 mg of dispase (Gibco-BRL) in 7.5 ml of DMEM/2% antibiotics-antimycotics] and kept at 4°C for 24 h. The following day the two tissue layers (epithelium and connective tissue) could be separated easily. Primary buccal epithelial cells (keratinocytes;

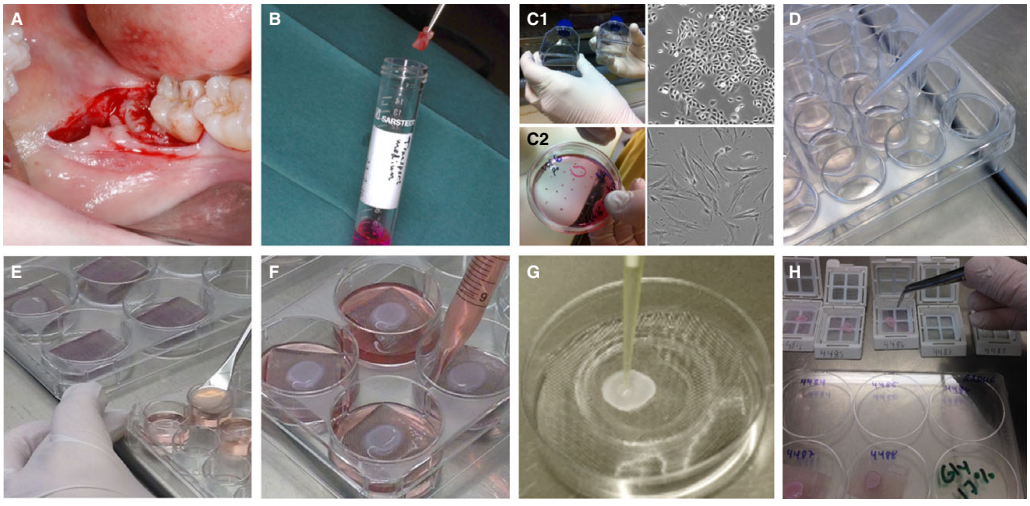

Fig. 1. Illustration of the key steps for reconstruction and exposure of the reconstructed normal human buccal mucosa (RNHBM) samples to glycerol. (A) Donor site. (B) Transfer of tissue to transport medium. (C) Subculture of keratinocytes (C^1) and fibroblasts (C^2). (D) Seeding keratinocytes on the fibroblast biomatrix. (E) Lifting the RNHBM samples to grids. (F) Adding medium during maturation. (G) Exposing. (H) Harvesting the RNHBM samples.

Fig. 1C[1]) were isolated through a combination of enzymatic digestion (trypsin EDTA × 10; Sigma) and mechanical separation of cells, and then cultured in keratinocyte serum-free medium (KSFM; Gibco-BRL) supplemented with 1 ng ml^{-1} of epidermal growth factor (EGF human recombinant; Gibco-BRL), 25 μg ml^{-1} of bovine pituitary extract (BPE; Gibco-BRL), 20 μg ml^{-1} of L-glutamine, and 1% AB/AM (100 U ml^{-1} of penicillin, 100 μg ml^{-1} of streptomycin, and 0.25 μg ml^{-1} of amphotericin B; Gibco-BRL). Primary buccal connective tissue cells (fibroblasts; Fig. 1C[2]) were isolated using an explant technique and cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Sigma), 20 μg ml^{-1} of L-glutamine, and 1% AB/AM (100 U ml^{-1} of penicillin, 100 μg ml^{-1} of streptomycin, and 0.25 μg ml^{-1} of amphotericin B). All cells were used in their third to fourth passages (split ratio of 1:4), at a viability of more than 80%, kept in a humidified atmosphere at 37°C, and supplemented with 5% CO_2.

Preparation of RNHBM samples

From each donor's biopsy, RNHBM samples were constructed when sufficient numbers of the two cell types – fibroblasts and keratinocytes – were achieved (Fig. 1D). The connective tissue equivalent was first reconstructed by mixing 350,000 fibroblasts per ml of collagen matrix: a mixture of collagen type I (REF354236, 3.81 mg ml^{-1}, LOT5061002; NAME OF COMPANY, Corning, NY, USA), reconstitution buffer, pH 8.15 [2.2 g of sodium bicarbonate ($NaHCO_3$), 0.6 g of sodium hydroxide (NaOH), 4.766 g of HEPES, in 100 ml of deionized H_2O (dH_2O)], and DMEM (Sigma) (19). A total of 400,000 keratinocytes in 1 ml of KSFM were seeded on top of each collagen matrix the following day, set as day 1. After 24 h, on day 2 of co-culture, the matrix, consisting of the two cell layers, was transferred to a metal grid placed in a well of a six-well plate (Fig. 1E). Three millilitres of fresh organotypic medium (OT-FAD) – DMEM/HAM's F12: 3/1 with 0.4 μg ml^{-1} of hydrocortisone, 5 μg ml^{-1} of insulin, 20 μg ml^{-1} of transferrin, and 50 μg ml^{-1} of L-ascorbic acid (all from Sigma) – was added underneath the metal grid along with 700 μl of conditioned medium. Throughout the experiment, care was taken to protect the RNHBM samples from spill of the medium. The RNHBM samples were allowed to mature for 9 days. Within this period, 2 ml of the medium was changed every second day (Fig. 1F). Emphasis was placed on acquiring enough cells to produce at least 12 RNHBM samples from each donor in order to obtain three samples for each of the three concentrations and the control. The number of RNHBM samples from each donor varied from 14 to 22. From all eight donors, a total of 134 RNHBM samples were cultivated. Of these RNHBM samples, 10 were either destroyed during cultivation or were not of satisfactory quality –six RNHBM samples turned upside down when lifted from the well-plate over to the grid and four RNHBM samples did not exhibit a sufficient number of keratinocytes. Hence, 124 RNHBM samples remained for further analyses. The RNHBM samples from each donor were allocated to one of the three concentrations or to dH_2O as a control (Table 1).

Exposure procedures to glycerol

On day 9 of co-culture (day 10 of culture and day 6 at the air–liquid interface), RNHBM samples were exposed to glycerol (batch no 17A121; Sanivo Pharma, Oslo, Norway) diluted in water to three different concentrations (Fig. 1G). At that time, a full-thickness, well-maturated buccal epithelium was formed. Based on an exploratory study (9) clinically relevant concentrations of 17%, 42.5% and 85% were chosen for exposure of RNHBM samples. A two-step procedure was used to decrease the surface tension and to obtain a more even exposure of the tissue surface without allowing any glycerol to spill into the culture medium

Table 1

Effect of exposure to water (control) and different glycerol concentrations on epithelial thickness, homeostasis (cell proliferation and apoptosis), and tissue integrity in in vitro *reconstructed normal human buccal mucosa*

Variable		No. of sections	*P*-value	ICC (95% CI)
Epithelial thickness (μm)		96		
Control (dH_2O)	88.85 (81.57–96.14)	28	<0.001[§]	0.03 (0.01–0.12)
17% glycerol	75.60 (68.81–82.39)	26	<0.001	
42.5% glycerol	110.09 (102.71–117.46)	20	<0.001	
85% glycerol	144.24 (137.10–151.37)	22	<0.001	
Proliferation* (positive cells per 1000 μm)		71		
Control (dH_2O)	5.85 (2.60–9.09)	17	<0.001[§]	0.52 (0.23–0.78)
17% glycerol	4.67 (1.46–7.90)	18	0.303	
42.5% glycerol	8.86 (5.64–12.08)	18	0.008	
85% glycerol	8.35 (5.14–11.57)	18	0.027	
Apoptosis[†] (positive cells per 1000 μm)		71		
Control (dH_2O)	1.05 (0.081–2.02)	17	<0.001[§]	0.23 (0.06–0.58)
17% glycerol	1.21 (0.26–2.17)	18	0.746	
42.5% glycerol	2.23 (1.27–3.18)	18	0.020	
85% glycerol	5.59 (4.64–6.55)	18	<0.001	
Tissue integrity[‡] (positive cell layers/negative cell layers)		71		
Control (dH_2O)	0.73 (0.61–0.84)	17	0.160[§]	0.28 (0.08–0.61)
17% glycerol	0.61 (0.49–0.73)	18	0.047	
42.5% glycerol	0.67 (0.56–0.79)	18	0.367	
85% glycerol	0.72 (0.61–0.84)	18	0.983	

*Ki-67; [†]Cleaved caspase 3; [‡]E-cadherin; [§]Test of homogeneity; dH_2O, distilled H_2O; ICC, intraclass correlation coefficient.

below. Initially, 60 μl of glycerol was placed on the RNHBM sample surface and sucked off. Immediately afterwards, a smaller volume (30 μl) of glycerol was applied, making a thin continuous film, and left on the tissue surface for 24 h. The same procedure was repeated for the controls, but with dH_2O. Exposed cultures were harvested on day 10 of the co-culture and immediately transferred to 4% buffered formalin, dehydrated, and embedded in paraffin (Fig. 1H). All experiments were run in parallel for the full range of glycerol concentrations and control (dH_2O), and the process was repeated for the RNHBM samples from each donor. Humidity in the incubator was measured throughout the whole procedure (Wood's hygrometer p-cv8005 Termohygrometer, Guelph, ON, Canada) and maintained at 89%–95%. All RNHBM samples were kept at the same middle level in the incubator.

Evaluation of the 124 sections

When evaluating the 124 sections in a light microscope, it was considered that 28 RNHBM samples from two of the donors had not developed an adequate epithelial layer. These samples were therefore excluded from further analyses. One section from each of the remaining 96 RNHBM samples was used for histomorphometry. Regarding immunohistochemistry, because of extremely resource-demanding procedures, a randomized selection of three RNHBM samples from each donor/concentration was used. For technical reasons, only two controls were used from one donor (Table 1, Figs 1 and 2).

Immunohistochemistry

For immunohistochemistry, 5-μm-thick formalin-fixed, paraffin-embedded tissue sections were cut and deparaffinized in xylene, hydrated through a graded alcohol series, and then rehydrated in dH_2O. For epitope retrieval, the tissue sections were microwave-treated in 10 mM citrate buffer, pH 6 (Dako, Glostrup, Denmark) or Tris/EDTA pH 9 (Dako) for 7 min at 950 W and then for 15 min at 350 W. After cooling for 20 min at room temperature, the specimens were incubated with primary antibodies in a humidified chamber at room temperature for 60 min. The markers used for cell proliferation, apoptosis, and tissue integrity

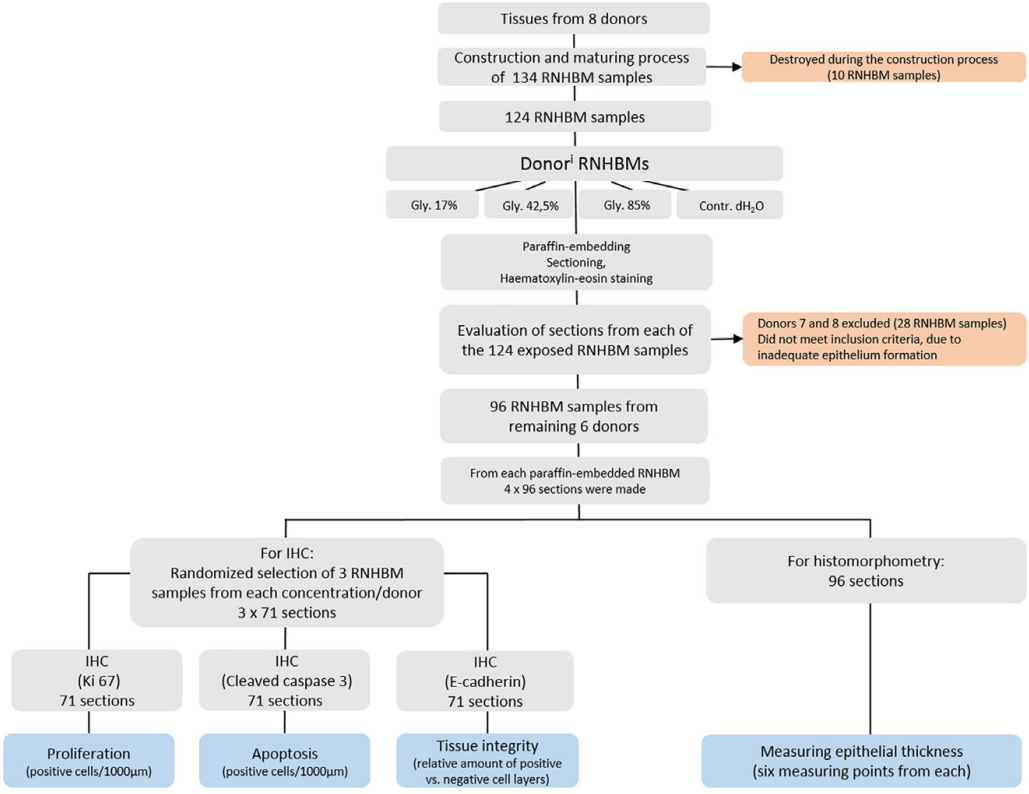

Fig. 2. Flow chart detailing the steps for construction of reconstructed normal human buccal mucosa (RNHBM) samples, and different stages in the histomorphometry and immunohistochemistry (IHC) analyses. dH_2O, distilled H_2O; Gly, glycerol.

were Ki-67 (M7240, Clone MIB-1, 1:300; Dako), cleaved caspase-3 (M3612, Clone NCH-38, 1:600; Dako), and E-cadherin (D175, Clone 5A1E, 1:3,000; Dako), respectively. The secondary antibodies were Dako REAL EnVision Detection System, Peroxidase/DAB+ and Rabbit/Mouse. Specimens incubated with antibody diluent only (Dako), or isotype-matched antibody (Dako) instead of primary antibody, were used as negative controls. As positive controls, either normal mucosa or RNHBM samples exposed to SLS from a previously published study (21) were used.

Evaluation of samples

Histomorphometry: Tissue sections (5 μm thick) from formalin-fixed tissues were cut, stained with haematoxylin & eosin (H&E) (Dako), and evaluated morphologically under a light microscope (Nikon Eclipse 80i; Nikon Instruments, Amsterdam, the Netherlands) at 200-fold magnification on six consecutive fields situated 200 μm apart. The outer 500 μm of the RNHBM samples was omitted from the measurements. Total epithelial thickness was measured as the distance from the surface of the epithelium to the epithelial tissue–connective tissue equivalent interface on a line perpendicular to the epithelial tissue–connective tissue equivalent interface.

Homeostasis (cell proliferation and apoptosis) and tissue integrity: To calculate the ratio of proliferating cells, the number of Ki-67-positive cells was determined in the basal and suprabasal cell layers, under a light microscope at 400-fold magnification. The average percentage of positive cells per 1,000 μm was counted for the whole length of the section, apart from the outermost 500 μm of the section. The ratio between apoptotic cells and the number of cleaved caspase-3-positive cells was obtained following the same procedure as described for Ki-67. Epithelial cell layers with cells expressing E-cadherin were counted and compared with non-exposed controls.

All measurements were made by the operator who was blinded to the exposure allocations.

Statistical analysis

Data are presented as mean and 95% CI. For comparison of the parameters across the different concentrations of glycerol, linear mixed effects models were applied. In these models, for each outcome, concentration was entered as a categorical fixed effect. Donor was included and controlled for in the model as a random effect accounting for the possible correlation between samples from the same donor. By including donor as a random effect, intraclass correlations (ICCs) for correlation of the measures from the same donor, for each of the parameters, can be calculated. An ICC close to 1 implies that samples from the same donor are highly correlated, while an ICC close to 0 implies that the samples can be considered as independent. The main results, based on the mixed models, were presented as estimated marginal mean values and mean differences, with 95% CI. All statistical analyses were performed using the statistical package STATA version 15 (Stata, College Station, TX, USA). Values of P <0.05 were considered statistically significant. A likelihood ratio test was used to test for homogeneity of the categories of the variable for each outcome.

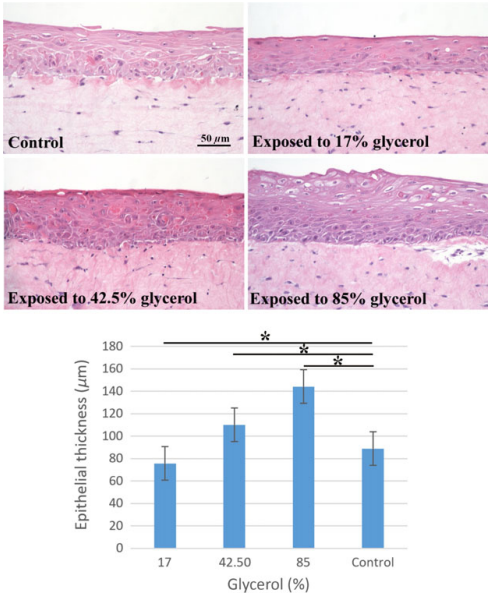

Fig. 3. Haematoxylin and eosin staining of representative three-dimensional (3D) reconstructed oral buccal mucosal tissues exposed to various concentrations of glycerol. Changes in epithelial thickness are shown (original magnification 200×; scale bar = 50 μm). In the bar chart, data are presented as mean ± SD, and statistically significant differences (P <0.05) are marked with *.

Results

Histomorphometric evaluation of the epithelial compartment in reconstructed tissues

Control RNHBM (i.e. tissues exposed to dH_2O only) displayed a well-differentiated, non-keratinized stratified squamous epithelium with an average epithelial thickness of 88.85 (95% CI: 81.57–96.14) μm. Exposure to 42.5% and 85% glycerol induced a significant increase in epithelial thickness ($P < 0.001$ and $P < 0.001$, respectively), whereas exposure to 17% glycerol induced a significant decrease in epithelial thickness ($P < 0.001$). No significant change in epithelial differentiation could be observed on H&E-stained sections at these higher glycerol concentrations. However, apoptotic bodies were frequently observed within the spinous cell layer at 42.5% and 85% glycerol, in contrast to tissues exposed to 17% glycerol and dH_2O (control). The ICC for measures of epithelial thickness was 0.03 (Table 1, Fig. 3).

Distribution and quantification of cell proliferation (determined by measuring expression of Ki-67)

Cell proliferation was most prominent along the basal cell layer and in the lower cell layers; and the superficial cell layers displayed no proliferation, either in controls or in exposed tissues. Exposure to the low concentration

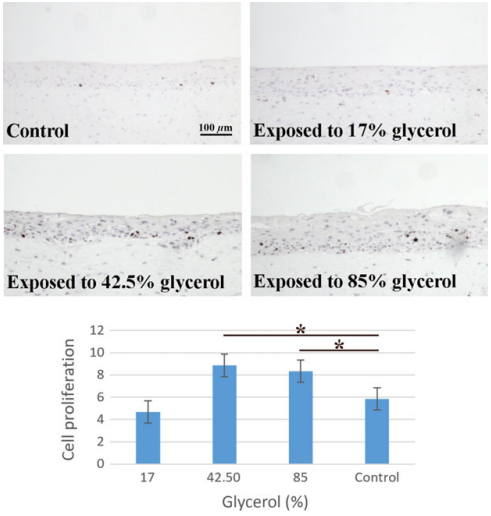

Fig. 4. Immunohistochemical staining of sections of representative three-dimensional (3D) reconstructed oral buccal mucosal tissues exposed to various concentrations of glycerol. Sections were stained for Ki-67, indicative of cell proliferation (original magnification 200×; scale bar = 100 μm). The bar chart shows quantification of cell proliferation, given as number of Ki-67-positive cells per 1000 μm length of epithelium–matrix interface. Data are presented as mean ± SD, and statistically significant differences (P <0.05) are marked with *.

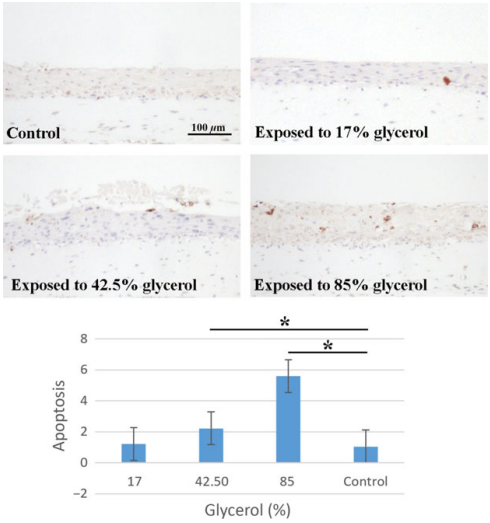

Fig. 5. Immunohistochemical staining of sections of representative three-dimensional (3D) reconstructed oral buccal mucosal tissues exposed to various concentrations of glycerol. Sections were stained for cleaved caspase-3, indicative of apoptosis (original magnification 200×; scale bar 100 μm). The bar chart shows apoptosis, given as number of cleaved caspase-3-positive cells per 1000 μm length of epithelium–matrix interface. Data are presented as mean ± SD, and statistically significant differences (P <0.05) are marked with *.

of glycerol (17%) did not induce significant changes in epithelial cell proliferation (as assessed by counting the number of Ki-67-positive cells per 1,000 μm; $P = 0.30$) when compared with controls. For the two higher concentrations of glycerol (42.5% and 85%), epithelial cell proliferation increased significantly ($P = 0.008$ and $P = 0.027$, respectively). The ICC for counts of Ki-67-positive cells was 0.52 (Table 1, Fig. 4).

Distribution and quantification of apoptosis (determined by measuring cleaved caspase-3)

The cleaved caspase-3 positively stained cells (apoptotic cells) were most frequent in the superficial cell layers in both control and exposed tissues. Exposure to the low concentration of glycerol (17%) did not induce a significant increase in the number of cleaved caspase-3 positively stained cells per 1,000 μm when compared with controls ($P = 0.75$). For the two higher concentrations of glycerol (42.5% and 85%), the number of apoptotic cells increased significantly ($P = 0.020$ and $P = 0.001$, respectively). The ICC for the counts of apoptotic cells was 0.23 (Table 1, Fig. 5).

Evaluation of tissue integrity (determined by measuring expression of E-cadherin)

The E-cadherin-positive cell layers formed a coherent belt from the basal cell layer to the stratum spinosum, both in controls and exposed tissues. Analysis of tissue integrity, evaluated as the relative number of E-cadherin-positive cell layers, showed a borderline significance ($P = 0.047$) between controls and tissues exposed to 17% glycerol. However, between controls and tissues exposed to glycerol at concentrations of 42.5% and 85%, there was no significant difference ($P = 0.37$ and $P = 0.98$, respectively). Test of homogeneity showed no overall statistically significant differences in the relative number of E-cadherin-positive cell layers ($P = 0.16$) between tissues exposed to the different glycerol concentrations. The ICC for the relative number of E-cadherin-positive cell layers was 0.28 (Table 1, Fig. 6).

Discussion

In the present study, we observed that glycerol concentrations of 42.5% and 85% induced an increase of apoptosis in RNHBM, which was visualized by immunohistochemistry using antibody detecting cleaved caspase-3. Cleaved caspase-3 was chosen as a marker for apoptotic cell death because it was shown in previous studies to be an easy, sensitive, and reliable method for detecting and quantifying apoptosis, compared with alternative methods such as the TdT-mediated biotin–dUTP nick-end labelling (TUNEL)

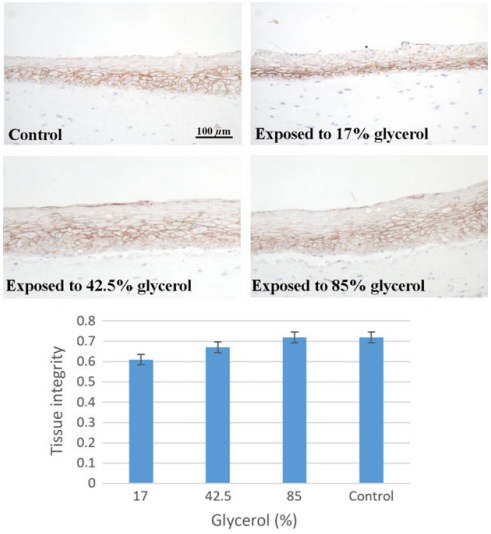

Fig. 6. Immunohistochemical staining of sections of representative three-dimensional (3D) reconstructed oral mucosal tissues exposed to various concentrations of glycerol. Sections were stained for E-cadherin, indicative of tissue integrity (original magnification 200×; scale bar = 100 μm). The bar chart shows tissue integrity, given as relative number of E-adherin-positive cell layers. Data are presented as mean ± SD, and statistically significant differences (P <0.05) are marked with *.

technique (22). The fact that an increase in apoptosis of individual cells is observed with exposure to glycerol indicates a certain toxic effect of glycerol at higher concentrations. The count of Ki-67-positive cells per 1,000 μm showed an increase in cell proliferation at the same high concentrations, indicating that glycerol is able to trigger a tissue reaction. The stimulation of cell proliferation is most probably a consequence and a response of epithelial tissue to the increased apoptosis induced by glycerol at high concentrations, in an attempt to maintain tissue homeostasis. Epithelial tissues maintain their homeostasis by balancing the continuous cell loss from desquamation on the surface with proliferation of cells in the basal cell layer. Therefore, increased proliferation, as a result of increased cell loss, is actually a natural response of a healthy epithelium in order to maintain tissue homeostasis (23).

Of concern in this study is the fact that the increase in cell proliferation was overbalancing apoptosis induced by exposure to glycerol, as indicated by the increase in epithelial thickness. For long-term exposure, one might argue that the use of high concentrations of glycerol could increase the risk of malignant transformation of the epithelium as it induces increased proliferation and thus increases the risk of accumulating DNA errors with each cellular replication. Nevertheless, the increased risk of glycerol exposure should be seen in a clinical context and with regard to the time perspective in palliative care patients. A possible effect of altered tissue integrity and changes in barrier function would have been more severe and more unfortunate with regard to penetration of pathogens. Such an effect does not seem to be present. This study shows that glycerol does not affect tissue differentiation or tissue integrity, as assessed by histological assessment of H&E-stained slides and immunostaining to visualize E-cadherin, a cell-to-cell adhesion molecule. The expression of E-cadherin was not changed by exposure to high concentrations of glycerol, indicating that the cell-to-cell contacts were not altered, indirectly suggesting a maintained barrier function. This is in contrast to the effects observed following exposure to SLS, in which E-cadherin expression was reduced and tissue integrity disrupted, as previously shown (21).

Using the mixed effects model, reporting ICC, we have also demonstrated that there are differences in the correlation between samples for the different parameters reported in this study. For epithelial thickness, each sample can be considered to be independent, even if they come from a limited number of donors. However, proliferation samples from the same donor are clearly dependent, and this has to be taken into consideration when interpreting the statistical analyses.

The RNHBM model has been chosen for its advantages in mimicking the native tissue, as mentioned in the Introduction. However, the use of an in vitro model obviously has some limitations. One may question whether it is appropriate to use healthy gingiva from young people, as the palliative care patients on whom glycerol is applied as a moisturizer are sick and often old. However, it is important to point out that the patient's mucosa is most often not diseased or damaged, and destruction and infection come as a result of dryness. Using seriously ill patients as donors for the RNHBM model would have been difficult for practical, ethical, and sampling reasons. Most dying patients are older than the donors in this study, and may have a mild epithelial atrophy. Even considering this aspect, in a previous study, the thickness of the RNHBM was shown to be less than normal mucosa (19). Another aspect that makes the RNHBM model appropriate is that its surface is dry and might therefore adequately replicate the mucosal surface of patients suffering from severe xerostomia. Formation of the salivary mucosal pellicle is provided by salivary mucins, probably mediated by hydrophobic interactions to the epithelial cell surface (24). Terminally ill patients with xerostomia often have an almost completely dry mucosal surface, perhaps even with the absence of a salivary pellicle.

Glycerol absorbs moisture from the air, even if the amount of moisture present is very small (8). As air humidity affects glycerol, emphasis was placed on controlling humidity in the incubator where the RNHBM samples were kept during maturation and exposure. The relative humidity in the incubator was 89%–95%, but dropped when opened. This is higher than the normal relative humidity, which is usually between 30% and 60%, and might have affected the glycerol

concentrations to become slightly lower than the baseline concentrations.

Glycerol is one of the most commonly used raw materials applied in cosmetics. It adds moisture by attracting water from the deeper layers of the skin (25). A similar system of water transport may be assumed when glycerol is applied to oral mucosa. In healthy persons, this would be of little consequence because of a continuous supply of liquid. In terminally ill patients, who are usually dehydrated (26), it seems likely that the mucosa might then be further desiccated when high concentrations of glycerol are used. However, it is not known whether these patients manage to replace fluid extracted from the underlying mucosa.

In conclusion, this study has shown that exposure of RNHBM samples to glycerol at high concentrations (42.5% and above) causes increased epithelial cell proliferation and epithelial thickness, as well as increased apoptosis compared with controls. Tissue integrity shows a reduced number of E-cadherin-positive cell layers of borderline statistical significance in tissues exposed to the lowest concentration of glycerol (17%), but no difference compared with controls at higher concentrations. Overall, these findings indicate that the use of glycerol is not harmful after short-term exposure but might pose certain risks after long-term exposure.

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Conflicts of interest – None to declare.

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Appendix Study I, II and III

Appendix I

Questionaire, Study I

I

Spørreskjema om palliativt munnstell

1) Sykehjem ☐ Sykehus ☐

2) Har dere prosedyrer for munnstell for døende pasienter på deres institusjon?

   Ja ☐ Nei ☐ Vet ikke ☐

3) Hvis ja, vennligst beskriv disse (evt. vedlegg prosedyrene):

   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____

4) Hvem har utarbeidet disse prosedyrene?

   _____
   _____

5) Føler du at man har tilstrekkelig kunnskap om dette emnet på deres institusjon?

   Ja ☐ Nei ☐ Vet ikke ☐

6) Opplever du at munnproblemer er et vesentlig problem for svært syke eller døende pasienter?

   Ja ☐ Nei ☐ Vet ikke ☐

7) Hvis ja, vennligst beskriv problemene (for behandler og/eller pasient):

   _____
   _____
   _____
   _____
   _____
   _____
   _____

Takk for hjelpen!

Appendix II

Informed Consent form, Study II

II

FORESPØRSEL OM DELTAKELSE I FORSKNINGSPROSJEKTET

Bruk av kunstig munnslimhinne for undersøkelse av opptak og reaksjon på midler brukt ved munnstell hos alvorlig syke eller døende personer

Dette er et spørsmål til deg om å delta i et forskningsprosjekt. Studiens hensikt er å undersøke ulike fuktighetsgivende midler som benyttes i stell av kreftpasienter og døende pasienter i norske helseinstitusjoner. Du ble spurt å delta i denne studien fordi du er frisk og vi har behov for slimhinne fra friske pasienter. Fra slimhinnen dyrkes enkeltceller som brukes til å lage en slimhinnemodell, som munnpleiemidlene skal testes på. Forskningsansvarlig for prosjektet er Universitetet i Bergen og Helse Bergen HF.

HVA INNEBÆRER PROSJEKTET?

En liten vevsprøve (3-4 mm) av munnslimhinnen tas under din planlagte operasjon i munnhulen. Prøven tas i operasjonsområdet. Området vil da være godt bedøvet (uten behov for ekstra bedøvelse). Prøvetakingen vil ikke få noen spesiell betydning i forhold til hvordan sårområdet normalt gror etterpå. Prøven får tildelt et nummer i den videre behandlingen, slik at de som behandler prøvene gjør dette anonymt.

Opplysninger som registreres om deg vil bli innhentet fra pasientjournalen ved behandlende klinikk (kjønn, alder, og eventuelle røykevaner). Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn har taushetsplikt.

MULIGE FORDELER OG ULEMPER

Metodene for vevsprøvetaking er klinisk etablerte, og det er utelukkende erfarne medarbeidere inkludert i studien. Vi ser ingen spesiell risiko eller ulempe ved å delta i prosjektet (ikke annen eller ekstra risiko enn det som er vanlig relatert til den aktuelle typen operasjon).

FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE SITT SAMTYKKE

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dette vil ikke få konsekvenser for din videre behandling. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte:

Professor Daniela Elena Costea, daniela.costea@uib.no , tel. 55973228 eller mobiltlf. 483 52 677 eller stipendiat, Siri Kvalheim, Siri.Kvalheim@uib.no, tlf. 55586488 eller mobiltlf. 481 73 272.

HVA SKJER MED OPPLYSNINGENE OM DEG?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet i hensikten med prosjektet. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene.

Alle dine opplysninger og prøver vil bli behandlet anonymt; det vil si uten navn, fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultater av studien når disse publiseres.

HVA SKJER MED PRØVER SOM BLIR TATT AV DEG OG INFORMASJON OM DEG?

Biopsiprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Haukeland Universitetssykehus. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken NanoMunnslimhinne. Professor Daniela Elena Costea er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare til 2018. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer. Biobanken opphører ved prosjektslutt.

ØKONOMI

Studien og biobanken er finansiert gjennom forskningsmidler fra EU og Universitet i Bergen.

GODKJENNING

Regional komité for medisinsk og helsefaglig forskningsetikk har vurdert prosjektet, og har gitt forhåndsgodkjenning (saksnr.2015/1851).

Du har rett til å klage på behandlingen av dine opplysninger til Datatilsynet.

KONTAKTOPPLYSNINGER

Dersom du har spørsmål til studien, kan du ta kontakt med Professor Daniela Elena Costea, daniela.costea@uib.no , tel. 55973228 eller mobiltlf. 483 52 677 eller stipendiat, Siri Kvalheim, Siri.Kvalheim@uib.no, tlf. 55586488 eller mobiltlf. 481 73 272.

JEG SAMTYKKER TIL Å DELTA I PROSJEKTET OG TIL AT MINE PERSONOPPLYSNINGER OG MITT BIOLOGISKE MATERIALE BRUKES SLIK DET ER BESKREVET

Sted og dato

Deltakers signatur

Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet

Sted og dato

Signatur

Rolle i prosjektet

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