Nanosafety aspects: Dental health care workers' perception of nanomaterials and *in vitro* nanotoxicity assessment using new approach methodologies

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Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This interdisciplinary study was performed at the Department of Clinical Dentistry and the Department of Clinical Medicine, Faculty of Medicine, University of Bergen and the Department of Pathology, Haukeland University Hospital, during the years 2016-2024. The main supervisor was Professor Anne Nordrehaug Åstrøm (Department of Clinical Dentistry, Faculty of Medicine, University of Bergen). Co-supervisors were Associate Professor Mihaela Cuida Marthinussen (Oral Health Centre of Expertise in Western Norway, Bergen), Professor Mihaela Roxana Cimpan (Department of Clinical Dentistry, Faculty of Medicine, University of Bergen) and Professor Daniela Elena Costea (Department of Clinical Medicine and Center for Cancer Biomarkers CCBio, Faculty of Medicine, University of Bergen; Department of Pathology, Haukeland University Hospital, Bergen). Biopsies were collected at the Department of Oral Surgery, Haukeland University Hospital. Reconstruction of 3D normal human buccal mucosa models and impedance-based monitoring of 2D cell cultures were performed at the Department of Clinical Dentistry, Faculty of Medicine, University of Bergen. Immunohistochemical analysis was performed at the Department of Pathology, Haukeland University Hospital, Bergen. Electron microscopy was performed at the Molecular Imaging Center at the Faculty of Medicine, University of Bergen.

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Abstract in English

Nanotechnology and its products are increasingly applied in many areas, such as medicine and dentistry, consumer products, energy, and agriculture. The increased use of nanomaterials (NMs) and nanoparticles (NPs) has raised concerns regarding their safety for humans and the environment due to some observed toxic effects of NPs. In this regard, it is important to provide efficient science-based risk communication. Dental health care workers have been using NMs for patient treatment. However, little is known about their perception of risk and benefits associated with NMs, as well as their intention to use such materials in the future. Concurrently, considerable progress has been made regarding the hazard and risk assessment related to NMs. However, the physicochemical characteristics responsible for the toxic effects of NMs and the mechanisms involved are still not completely elucidated. There is an urgent need to develop new approach methodologies that are biomimetic and less prone to NM-induced interferences, which are more relevant for human exposure and reduce the uncertainty of hazard and consequently, risk assessment.

The overall aim of the present study was to address the issue of nanosafety in dentistry from two different perspectives: (1) assessment of risk/benefit perceptions and intention to use NMs by dental professionals and (2) evaluation of *in vitro* cytotoxic effects of TiO_2 NPs related to oral and lung exposure using new approach methodologies (NAMs).

Electronically administered questionnaires were distributed to a census of 1792 dentists and dental hygienists employed in the Public Dental Health Service (PDHS) in Norway. In **Study I**, covariates of risk and benefit perceptions were explored using binary logistic regression analysis. In **Study II,** the augmented Theory of Planned Behavior (TPB) was employed to assess factors predicting intention to use dental NMs using structural equation modeling technique. In **Study III,** primary human normal oral fibroblasts, lung cancer epithelial cells (A549) in conventional two-dimensional (2D) monolayers, and three-dimensional (3D) reconstructed normal human buccal mucosa (RNHBM) models were exposed to rutile spherical (40 nm) and spindle-shaped (40×10 nm) TiO_2 NPs at different concentrations. Impedance-based monitoring was used to

assess the viability of cells in monolayers and immunohistochemistry was used to assess the proliferation, apoptosis and tissue integrity of RNHBM epithelium.

A total of 851 dental health care workers responded, providing a response rate of 47.5%. **Study I** revealed that more than half of the Norwegian dental health care workers had high levels of benefits and risks perceptions associated with the use of dental NMs. Feeling safe to use NMs and being worried about their increasing use were the strongest covariates of perceived benefits and risks, respectively. It was also revealed that dental health care workers had moderate knowledge about the use of NMs in dentistry. **Study II** demonstrated that the strongest predictors of intention to use dental NMs were attitudes and perceived behavioral control, followed by past behavior and subjective norms. Risk perception had an indirect negative effect on intention through attitudes, past behavioral control and subjective norms, implying that high risk perception was associated with low intention to use NMs. **Study III** has shown that exposure to both types of TiO_2 NPs led to a decrease in the proliferation of primary normal oral fibroblasts, but not of lung cancer epithelial cells. The proliferation, apoptosis and tissue integrity of 3D oral mucosa models were not significantly affected by the TiO_2 NPs.

The survey revealed several important factors associated with the risk and benefit perceptions and intention to use dental NMs, which should be considered by policy makers for risk communication and management of NMs in dental health care services. The *in vitro* study raises concerns due to observed impaired growth of oral fibroblasts after 24 h of exposure to the selected TiO_2 NPs. The NAMs employed in this work, *i.e*., *in vitro* 3D oral mucosa models and impedance-based toxicity screening, represent promising tools for nanotoxicity assessment.

Abstract in Norwegian

Nanoteknologi er i utstrakt bruk innen sektorer som medisin og odontologi, forbrukerprodukter, energi og landbruk. Toksiske effekter knyttet til bruk av nanomaterialer (NM) og nanopartikler (NP) har vekket bekymring på materialenes sikkerhet for mennesker og miljøet. Effektiv risikokommunikasjon krever informasjon om hva brukerne mener om ny teknologi. Tannhelsepersonell har brukt nanomaterialer til pasientbehandling, men man vet lite om deres kunnskap, holdninger, nytte- og risiko opplevelse knyttet til nanomaterialer og deres intensjon om å bruke slike materialer i fremtiden. Til tross for betydelige fremskritt når det gjelder risikovurderinger, er kunnskap om nanomaterialers fysiske og kjemiske egenskaper fortsatt mangelfull. Det er et stort behov for å utvikle nye metoder som er biomimetiske, mindre utsatt for NM-induserte interferenser, mer relevante for menneskelig eksponering og som reduserer usikkerheten rundt fare- og risikovurdering.

Målet med denne studien er (1) å undersøke risiko/nytteoppfatninger og intensjonen om å bruke nanomaterialer blant tannleger og tannpleiere i Norge og (2) å undersøke *in vitro* cytotoksiske effekter av TiO_2 NP ved bruk av nye tilnærmingsmetodologier.

I 2017 ble det gjennomført en survey undersøkelse der elektronisk administrerte spørreskjema ble delt ut til en sensus av 1792 tannleger og tannpleiere ansatt i Den offentlige tannhelsetjenesten i Norge. I **studie I** ble risiko- og nytteoppfatninger assosiert med bruk av nanomaterialer identifisert ved hjelp av binær logistisk regresjonsanalyse. I **studie II** ble den utvidede teorien om planlagt atferd (TPB) brukt for å vurdere faktorer som predikerer tannhelsepersonellets intensjon om å bruke dentale nanomaterialer. Strukturell ligningsmodelleringsteknikk ble brukt til å analysere data. I **studie III,** som er basert på laboratorieeksperimenter**,** ble primære orale fibroblaster, lungekreftepitelceller (A549) og 3D rekonstruerte normale menneskelige munnslimhinnemodeller eksponert for rutil sfæriske (40 nm) og spindelformede (40 × 10 nm) TiO_2 NP i forskjellige konsentrasjoner. Impedansbasert monitorering ble brukt for å vurdere overlevelse av celler i to-dimensjonal monolag og

immunhistokjemi ble brukt til å vurdere proliferasjon, apoptose og vevsintegritet av tre-dimensjonale oralmunnslimhinne modeller.

Totalt deltok 851 tannleger og tannpleiere i surveyundersøkelsen i 2017 (svarprosent 47.5%). **Studie I** viste at mer enn halvparten av norske tannleger og tannpleiere hadde høye nivåer av nytte- og risikooppfatninger knyttet til bruk av dentale NM. Å føle seg trygg på bruken av NM og å være bekymret for økende bruk hadde den sterkeste sammenhengen med bruk av NM. Videre viste studien at tannhelsepersonell hadde moderat kunnskap angående bruk av dentale nanomaterialer i pasientbehandling. **Studie II** bekreftet hypotesene i TPB og viste at de sterkeste prediktorene for intensjon om å bruke dentale nanomaterialer var positive holdninger og opplevelse av atferdskontroll, mens tidligere bruk av NM hadde mindre betydning. **Studie III** har vist at eksponering for begge typer TiO_2 NP førte til en redusert proliferasjon av orale fibroblaster, men ikke av lungekreftepitelceller. Proliferasjon, apoptose og vevsintegritet til 3D munnslimhinne modeller ble ikke signifikant påvirket av TiO_2 NP.

Studien har avdekket flere faktorer knyttet til risiko- og nytteoppfatninger og til intensjon om å bruke dentale nanomaterialer blant norske tannleger og tannpleiere. Resultatene kan være til nytte for beslutningstakere ved kommunisering av risikoinformasjon og når det gjelder håndtering av nanomaterialer i tannhelsetjenester. Resultatene fra *in vitro* studien vekker bekymring på grunn av observert nedsatt vekst av orale fibroblaster etter 24 timers eksponering for de utvalgte TiO_2 NP. De nye tilnærmingsmetodologier som ble brukt i denne studien, det vil si *in vitro* 3D munnslimhinnemodeller og bioimpedansbasert cellemonitorering, representerer lovende verktøy for vurdering av nanotoksisitet.

Abbreviations

AIDS	Acquired immune deficiency syndrome
ATCC	American type culture collection
CFA	Confirmatory factor analysis
CFI	Comparative fit index
CI	Cell index
COVID-19	Coronavirus disease 2019
DCFH-DA	2',7'-dichlorofluorescein-diacetate
DHE	Dihydroethidium
DLS	Dynamic light scattering
DMEM	Dulbecco's modified eagle's medium
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ELS	Electrophoretic light scattering
FAD-OT	
FBS	Fetal bovine serum
FIML	Full information maximum likelihood
HA	Hydroxyapatite
H&E	Hematoxylin and Eosin
HD	Hydrodynamic diameter
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
ICC	Intraclass correlation coefficient
KSFM	Keratinocyte serum-free medium
MBCl	Monochlorobimane
MECD	Microscopy equivalent circle dimatere
MLR	Maximum likelihood estimator with robust standard errors
MTS	(3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2*H*-tetrazolium
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide)
NAM	New approach methodology
nm	Nanometer
NM	Nanomaterial
NP	Nanoparticle
PBC	Perceived behavioral control
PBS	Phosphate-buffered saline
PDI	Polydispersity index
RMSEA	Root mean square error of approximation
RNA	Ribonucleic acid
RNHBM	Reconstructed normal human buccal mucosa
ROS	Reactive oxygen species
RTCA	Real-time cell analysis
SEM	Structural equation modelling
SN	Subjective norms

SRMR	Standardized root mean square residual
TEM	Transmission electron microscope
TPB	Theory of planned behavior
TRA	Theory of reasoned action
WST-8	(2-(2-Methoxy-4-nitrophenyl)-3-(4nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium
yo	Years old

List of Publications

I. **Xenaki, V.**, Costea, D. E., Marthinussen, M. C., Cimpan, M. R., and Astrom, A. N. (2020): Use of nanomaterials in dentistry: covariates of risk and benefit perceptions among dentists and dental hygienists in Norway, *Acta Odontologica Scandinavica,* Vol. 78: 152-60.

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III. **Xenaki V.**, Xue Y., Lie S. A., Marthinussen M. C., Åstrøm A. N., Costea D. E., Cimpan M. R. (2024): Effect of TiO_2 nanoparticles on cell death and proliferation: An *in vitro* study on 2D and 3D biological models, *Manuscript*

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Xenaki, V, Marthinussen, M.C., Costea, D. E., Didilescu, A.C., Susin; C., Cimpan, M. R., and Åstrøm, A.N. 2020. 'Knowledge about nanotechnology and intention to use nanomaterials: A comparative study among dental students in Norway and Romania', *European Journal of Dental Education*, 24: 79-87.

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Contents

1. Introduction

The topic of nanosafety is a subject of ongoing debate. Although nanotechnology has become a part of our daily life, the question of its safety for humans and the environment is not fully answered [1, 2]. It was discovered that materials that are inert in their bulk form, could exhibit toxic potential in the nanoform. For example, titanium-based implants have been widely used in medicine and dentistry due to their high biocompatibility and inertness. However, according to *in vitro* and *in vivo* tests, TiO_2 in nanoform has demonstrated cyto- and genotoxicity [3-6]. Several challenges in nanotoxicity assessment, such as NM interferences with reagents and detection systems, insufficient physicochemical characterization, and lack of standardization, contribute to uncertainty regarding hazard and risk assessment and decision making [7]. At the same time, lack of consensus regarding nanosafety may result in fluctuation of public opinion and opposition to nanotechnology. In this respect, it is important to provide reliable and balanced information about potential benefits and risks associated with the use of NMs.

This thesis addresses safety aspects of dental NMs as studied from two perspectives – (1) assessment of dental health care workers' risk and benefits perceptions of NMs and their intention to use such materials and (2) *in vitro* evaluation of selected TiO_2 NPs toxicity on two-dimensional (2D) cells in monocultures and on three dimensional (3D) buccal mucosa models using NAMS.

1.1 The concepts of nanotechnology, nanomaterials, nanoparticles, nanosafety and NAMs

Nanotechnology is an interdisciplinary field combining engineering, chemistry, physics, biology and medicine [8]. It is relevant for many sectors, such as chemicals, consumer products, health, energy, and the environment [9]. Nanotechnology has brought advancement to multiple industries, and it is envisaged to make significant impact on human society and environmental sustainability [10].

Nanomaterials have been recently defined by the European Commission [11] as "natural, incidental or manufactured materials consisting of solid particles that are present either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50% or more of these particles in the number-based size distribution fulfil at least one of the following conditions:

a) one or more external dimensions of the particle are in the size range from 1 nm to 100 nm;
b) the particle has an elongated shape, such as rod, fiber or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;
c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm."

As suggested by Auffan et al. (2009), inorganic particles that have at least one dimension less than 100 nm are defined as **nanoparticles** (NPs) [12]. Due to their small size NPs possess a range of unique properties that make them advantageous over larger particles of the same material. However, much smaller size and higher surface-to-volume ratio of NPs can make them more toxic compared to bulk material as they can more easily penetrate cells and biological barriers and accumulate in different organs [13-15].

Nanoparticles can be classified by origin as natural, accidental and engineered [16]. Natural NPs are found in nature – ashes, viruses, small molecules, while accidental are created unintentionally as byproducts of certain processes. Engineered NPs are produced by humans with intention and can vary in size, shape, composition, porosity, phase, solubility, uniformity, etc. [16, 17]. Engineered NPs can further be classified as organic, carbon-based and inorganic [18].

Nanoparticles are seldom present as single particles as they tend to agglomerate when they come in contact with water, cell culture medium or biological fluids [19]. An **agglomerate** is an assembly of weakly bound particles and/or aggregates whose total surface area is similar to the sum of the surface areas of the individual components. In

contrast, an **aggregate** is an assembly of strongly bound primary particles that are fused together. The total surface area of an aggregate is usually smaller than the sum of the surface areas of the individual particles [20, 21].

Nanosafety refers to the evaluation of toxicity level of engineered NMs, and assessment of risks related to the use these NMs to human health and the environment [22].

New approach methodologies (NAMs) are defined as alternative and/or complementary methods to conventional animal testing used to assess hazardous properties of chemicals. NAMs include a wide range of *in silico*, *in vitro*, *ex vivo* and *in chemico* approaches, which follow the 3R principle of replacing, reducing, and refining of animal experiments [23].

1.2 Use of nanomaterials in medicine and dentistry

Nanotechnology is envisaged to bring great advancements in the field of medicine and dentistry by providing innovative solutions to unsolved medical problems [20]. The field of nanomedicine has started to evolve only few decades ago and has already achieved promising results. Medical applications of NMs include diagnosis, prevention, monitoring, control and treatment of diseases [20]. More than 90 nanomedicines have been approved by U.S. Food and Drug Administration and European Medicines Agency [24-26]. Nano-based imaging is used for cancer diagnostics by utilizing superparamagnetic iron oxide NPs, fluorescent silica dots and fluorescent polymeric NPs [27]. Treatment of cancer and infectious as well as non-infectious diseases has benefited from using drug nanocrystals and polymeric NPs [25, 26]. Nanotechnology has also been used in the development of vaccines. A recent example is the fabrication of RNA-based COVID-19 vaccine, where lipid NPs were used in order to protect and deliver RNA to the cells [28-32]. Moreover, extensive research is done regarding nanotechnology-based regenerative medicine and tissue engineering [33].

Nanotechnology has brought numerous advancements in the field of dentistry [34]. Nanosized silica particles have been added to dental composites (restorative tooth filling materials) resulting in the production of nanofillers and nanohybrids [35, 36]. The load of the particles per mass or volume is bigger in nanofilled then in microfilled composites, which determine their superior properties, such as less curing shrinkage, better handling, improved esthetical characteristics and better wear resistance [35-37]. A great variety of nanocomposites is available on the market, such as Filtek Supreme (3M ESPE, St Paul, USA), Grandio (VOCO, Cuxhaven, Germany), Ice (SDI, Bayswater, Australia Australia), to name a few [38]. Dental adhesive systems have been used in dentistry to promote bonding between tooth structures and restorative material. Adhesives with silica nanofiller particles have exhibited improved bonding strength and reduced microleakage, which results in better adhesion and decreased risk of secondary caries [39, 40]. Commercially available adhesives with NPs include Adper™ Scotchbond™ (3M ESPE, St. Paul, USA), Clearfil™ SE Protect Bond (Kuraray Noritake Dental, Tokyo, Japan), OptiBond Solo Plus (Kerr, Orange, CA, USA) and others [41]. Nanoparticles have been successfully used to treat bone defects in dental settings. Hydroxyapatite (HA) is a naturally occurring form of calcium phosphate that constitutes the largest part of inorganic components in human bones and teeth [42]. Nanocrystalline-HA has been used to treat bone defects and enamel defects, as it has shown the ability to induce bone regeneration and promote enamel remineralization [35]. Various NPs (nano-HA, TiO_2, carbon-based nanodiamonds) have been used for dental implant coating, as they can improve osseointegration, prevent corrosion and subsequent loss of implant [35, 43]. Metals and their oxides (sliver, zinc) with antibacterial effects have been commonly used in dental materials. Recent research has shown that the same materials at the nanolevel exhibit better antibacterial properties due to larger surface area of NPs and the ability to directly interact with bacterial cell wall due to small size [37]. Thus, metal NPs (silver, TiO_2 and ZnO) have been applied in oral care products, dental filling materials, endodontic sealants (root canal filling materials) and dentures. Besides the abovementioned examples, a great number of dental NMs have been developed and tested in the lab,

demonstrating enhanced properties compared to conventional materials [44, 45]. However, long-term clinical studies are needed to confirm the laboratory findings.

1.3 A social cognition approach to the study of using nanomaterials in dentistry

1.3.1 The theory of planned behavior

The theory of planned behavior (TPB), together with the health belief model, health locus of control, protection motivation theory and social cognitive theory, belongs to the social cognition models [46]. Social cognitions are concerned with how people process and respond to social information implying that social and health related behaviors are best understood as a function of people's perception of reality [47]. Among all the factors determining health behaviors, cognitive factors are recognized as very important, since they are amenable to change and differ between individuals from the same socio-demographic background. Hence, a great deal of research has focused on studying cognition factors to understand why individuals perform various behaviors. Social cognition models provide an important framework for identification of cognitive factors that explain health related behaviors. In other words, it gives basis for the planning and implementation of interventions aimed to improve individuals' and populations' health and oral health [46].

The TPB and its precursor, the theory of reasoned action (TRA), is among the most commonly used social cognition models. The TRA was elaborated in order to explain volitional behavior [48]. TRA suggests that attitudes towards a particular behavior influence the performance of the behavior through intention (Figure 1). Attitudes are considered a person's overall evaluation of behavior as good or bad and intention is a persons' conscious decision or motivation to engage in a particular behavior [48, 49]. The stronger the person's intention, the greater the probability that the behavior will be performed. In turn, intention is a function of attitudes towards behavior and subjective norms. Attitudes and subjective norms are determined by a set of underlying beliefs. Attitudes are underpinned by beliefs about positive and negative consequences of performing the behavior, whereas subjective norms are underpinned by normative

beliefs about whether significant others approve or disapprove his/her engagement in the behavior. In other words, subjective norms assess the perceived social pressure to perform or not to perform the behavior. According to the TRA, behavior is under the control of intention. Thus, this approach could only be applied to volitional behaviors.

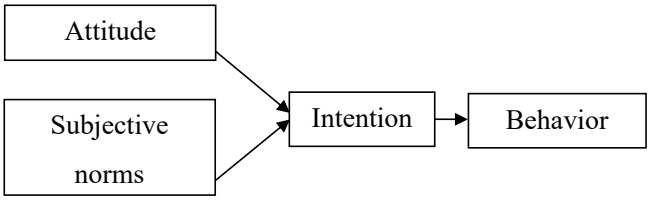

Figure 1. The theory of reasoned action [48].

In an attempt to broaden the TRA applicability to predict non-volitional behaviors, Ajzen suggested to incorporate perceived behavioral control (PBC) [50]. Actual control, in sense of available resources and opportunities, is important for the performance of behavior. But of greater interest is the perception of behavioral control, which is an individual's expectancy that the behavior is under his/her control [51]. Thus, according to the TPB, behavior is a joint function of intention (which is a function of attitudes and subjective norms) and PBC (Figure 2). In other words, individuals are likely to follow a particular behaviour if they believe that the behavior have positive consequences (positive attitude), if they believe that the important others would approve the performance (subjective norms) and if they feel to have necessary resources to perform the behavior (PBC) [49]. The relative importance of attitudes, subjective norms and PBC is expected to vary according to the specific behaviour and study group [50].

Apart from attitudes, subjective norms and PBC, the TPB is open to external predictors. It is assumed that the impact on intention from external variables is mediated through the constructs of the TPB model (Figure 2). Empirical works have shown that among external factors, self-identity, past behavior, knowledge and trust are the most used predictors of intention [52-55].

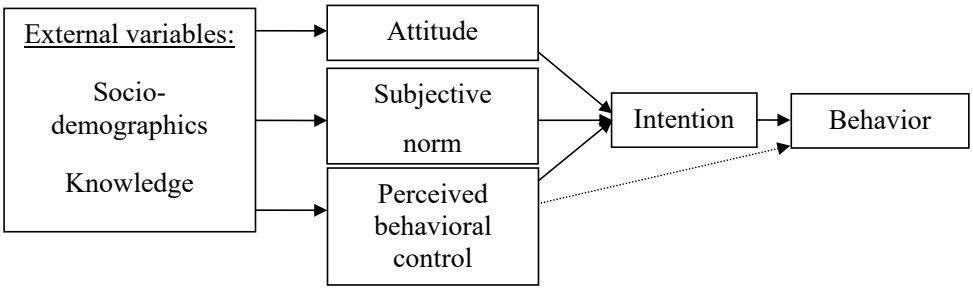

Figure 2. The theory of planned behavior (Ajzen 1991).

The TPB has been proven to be a useful tool in predicting and explaining various behaviors and has been successfully applied in occupational, environmental, educational, business and management research [46, 51, 56]. A recent review by Bosnjak, Ajzen and Schmidt have shown that the TPB was applied in more than 4,200 papers (as of April 2020) and it was mostly used in "public environmental occupational health" domain [56]. The theory has also been used to study various occupational behaviors among dental health care workers (Table 1). However, to our knowledge, this theory has not been applied to study dental health care workers' behaviors related to nanotechnology.

Table 1. Studies (n = 14) utilizing TPB to explain occupational behavior among dental health care workers from 1999 until 2020.

Study	Country	Study population	Target behavior	Main findings
Godin et al. 1999 [57]	Canada	Dentists (n = 771)	Intention to provide dental care to HIV^+ and AIDS patients	The main predictors of intention were perceived behavioral control, personal normative belief and habit of treating HIV^+ and AIDS patients
Bonetti et al. 2006 [58]	Scotland	General dental practitioners (n = 214)	Taking intra-oral radiographs	PBC was the strongest prediction of intention, followed by attitudes. SN was not a significant predictor of intention
Bonetti et al. 2009 [59]	Scotland	General dental practitioners (n = 133)	Placing of fissure sealants and intention to place fissure sealants	Behavior was predicted by intention, attitude and PBC in descending order. Intention was predicted by attitudes and PBC. SN was not a significant predictor of neither behavior nor intention
Bonetti et al. 2010 [60]	Scotland	General dental practitioners (n = 120)	Decision to place preventive dental sealants	Attitudes and perceived behavioral control predicted intention
Leavy et al. 2014 [61]	Scotland	Dentists working in primary dental care (n = 124)	Reporting occupational exposures to blood and other oral fluids in Scottish dental practices	Dentists' intention to report patient exposures was significantly higher than their own exposure. Dentists did not think reporting exposures would result in their colleague/patient losing faith in their competence. Reporting was perceived an easy procedure.
Pollack et al. 2014 [62]	USA	Dentists (n = 1802)	Willingness to conduct HIV screening in the dental care setting	57% of participants are willing to offer HIV testing. Normative influences were strongly associated with decreased willingness.
Yusuf et al. 2016 [63]	England	Dentists working in primary dental care (n = 164)	Preventive behaviors (asking and advising on diet, tobacco and alcohol consumption)	Attitudes were the strongest predictor, followed by perceived behavioral control. Subjective norms did not predict preventive behaviors.
Tantawi et al. 2018 [64]	Algeria, Egypt, Jordan, Kuwait, Libya, Palestine, Saudi Arabia, Yemen	Dentists (n = 2936)	Intention to report suspected violence	Attitudes had the greatest impact on intention, followed by subjective norms and perceived control.

Table 1 (continued). Studies (n = 14) utilizing TPB to explain occupational behavior among dental health care workers from 1999 until 2020.

Study	Country	Study population	Target behavior	Main findings
Tantawi et al. 2019 [65]	Saudi Arabia	Dentists (n = 255)	Intention to manage drug users	Perceived norms had the strongest association with intention, followed by perceived control. Attitudes were not significantly associated with intention.
Brattabo et al. 2019* [66]	Norway	Dentist and dental hygienists (n = 1200)	Intention to report suspected child-maltreatment	Instrumental attitudes and perceived behavioral control were the strongest predictors of intention
Singer et al. 2019 [67]	USA	General dentists (n = 1802)	Willingness to provide a finger stick test to support screening for cardiovascular disease risk in dental care setting	Less than 50% were willing to provide finger test. Willingness was greater for dentist who were currently screening for hypertension with blood pressure cuff, whose health history forms included questions about obesity and who agreed that their role as health care professionals should include screening for hypertension.
Aliakbari et al. 2020 [68]	Iran	General dentists and dental specialists (n = 63)	Improving health and ergonomic position	Subjective norm, perceived control, attitude and behavioral intention were predictors of intention
Ammar et al. 2020 [69]	28 countries	Dental academics (n = 1862)	Frequent handwashing and avoidance of crowded places	Greater fear of infection, worries about professional responsibility and worries because of restricted mobility was associated with more frequent handwashing and more avoidance of crowded places
Shubayr et al. 2020 [70]	Saudi Arabia	Dental health care workers (324)	Prevention and control of COVID-19 infection	Attitudes and subjective norms were significant predictors of intention

* The study has utilized reasoned action approach

1.3.2 Attitudes and risk/benfit percptions regarding nanomaterials

According to Ajzen and Fishbein, attitude is “an evaluation of an object, concept, or behavior along a dimension of favor or disfavor, good or bad, like or dislike” [71]. Attitudes towards a behavior are based on perceived positive and negative consequences of performing that behavior such as perceived benefits and risks. In contrast to attitudes towards performance of a particular behavior, attitudes towards the object of nanotechnology or NMs are more commonly found in the research literature.

To date, a significant body of research has studied attitudes towards nanotechnology and NMs by laypersons, experts, risk assessors and risk managers in different countries [72-81]. Across these studies, several important factors influencing public attitudes towards nanotechnology were identified such as knowledge, trust in stakeholders, media representation, religious beliefs and demographics [80, 81]. Generally, it was demonstrated that lay persons have mostly neutral or positive attitudes towards nanotechnology [72, 77, 79]. However, compared to experts, lay persons exhibited higher risk perceptions of NMs, except for some issues related to potential environmental risks and long-term health effects, that raised higher concerns among scientists than among non-experts [80, 82]. Recent studies have demonstrated that scientists have different risk perceptions depending on the field of study they belong to. Thus, "upstream" scientists (working with the development of new NMs) perceived little or no risks related to nanotechnology, while "downstream" scientists (who investigate the environmental and health effects related to NMs) emphasized potential new hazards [83]. Trust in industry leaders, risk managers and policy makers is a strong predictor of positive attitudes towards nanotechnology. It was demonstrated that higher trust is associated with higher benefits and lower risk perceptions as well as greater acceptance of the novel technology [72-76, 78, 81]. It has been shown that the general public has higher trust in people working in nanotechnology industries and nanotechnology researchers than in governmental agencies and journalists, indicating that scientists could be the most appropriate group to communicate risks to the public [73, 76]. Some authors found that the degree of religiosity is associated with acceptance and support of nanotechnology, reporting that people with strong religious beliefs were less likely to morally accept nanotechnology [84, 85]. However, other researchers found that individual religiosity was weakly related to nanotechnology attitudes [86, 87]. Demographic factors also play an important role in public perception of nanotechnology. Men, people with higher education and higher income were found to give greater support for emerging technologies [75, 81, 87-91].

The general public has demonstrated limited knowledge about nanotechnology [77, 81, 87, 92-94]. Moreover, studies among university students have also revealed their

limited knowledge [95, 96]. However, according to the report of the European Chemical Agency, there is a steady growth of awareness about nanotechnology among European residents. The average percentage of respondents who were aware of NMs has increased from 45% in 2005 to 65% in 2020 [97]. Knowledge is considered to have an impact on attitudes towards nanotechnology [80]. Some studies demonstrated that higher degree of knowledge was associated with positive attitudes towards NMs [72, 84, 98], while in others there was no association [79, 99]. For instance, it was shown in a longitudinal study that a moderate increase in knowledge over time did not lead to more positive attitudes towards nanotechnology [99]. On the contrary, people who were found to be unfamiliar with the concepts of nanotechnology still had positive attitudes towards it. It was suggested that scientific literacy in general, rather than knowledge about nanotechnology specifically, is a predictor of positive perception of this novel technology [75].

1.4 *TiO_2 nanoparticles and in vitro putative toxic effects*

1.4.1 TiO_2 Nanoparticles

TiO_2 is a transitional metal oxide that mainly exists in three crystalline structures – anatase, brookite and rutile, with the last one being the most common and stable form [100]. It possesses a range of beneficial properties, such as high chemical stability and high photoactivity as well as resistance to corrosion, oxidation and high temperatures [101]. All these features make it the most produced material at the nano-level [4, 102] with a wide range of applications, such as cosmetic industry, paper, plastic and rubber industry, wastewater treatment, air purification, construction, agriculture as well as pharmaceuticals, medicine and dentistry [103, 104].

In medicine, TiO_2 NPs were tested in phototherapy for cancer treatment, drug delivery, cell imaging, tissue and genetic engineering, to name a few [101, 103]. In dentistry, TiO_2 NPs are used for surface coating of dental implants to overcome failures in relation to mechanical and biological factors. They were also added to dental composites to enhance their wear resistance and to dental adhesives to improve their bond strength [105, 106]. Besides that, TiO_2 NPs were demonstrated to enhance

antibacterial properties of dental composites, which is especially beneficial in orthodontics to prevent caries lesions related to fixed orthodontic appliances [107, 108]. In addition, TiO_2 NPs were shown to have a positive effect on sensitive teeth by occluding dentine tubules [109, 110].

1.4.2 Exposure to TiO_2 nanoparticles

Humans can be exposed to engineered NPs during both manufacturing process and use of products containing NPs, such as cosmetics, toothpaste, sunscreen, food, pharmaceuticals, and dental materials. The possible routes of NPs exposure are inhalation, ingestion, dermal penetration, and injection [111, 112]. It was also shown that Ti and TiO_2 NPs can be released from titanium-based hip or dental implants, into the surrounding tissues [113-115].

Inhalation of TiO_2 NPs is common for occupational exposure. The recommended limit for this exposure is set to 0.3 mg/m^{-3} for up to 10 h working shift per day during 40 h working week [116, 117]. Consumers can also be exposed to TiO_2 NPs, when using cosmetic products in spray or powder form. According to the Scientific Committee on Consumer Safety, the estimated amount of TiO_2 NPs that can potentially be absorbed per day is 6.3×10^{-5} mg/kg bw (bw 61 kg) [118].

Although the measurement of human exposure to TiO_2 NPs via ingestion is a difficult task, attempts have been made to quantify oral intake of TiO_2 NPs originating from food (dairy products, sweets, chewing gums, pastries) and toothpaste [119-121]. The results vary between countries due to differences in dietary habits. In Netherlands, the oral intake in adults was evaluated to be quite low (0.06-0.17 mg/kg/bw/day) as opposed to the intake in the United States (0.2-0.7 mg/kg/bw/day), the United Kingdom (1.0 mg/kg/bw/day) and Germany (0.5-1.0 mg/kg/bw/day) [119]. Children are reported to have 2-3 times higher oral intake compared to adults because of low body weight and high consumption of sweets which contain TiO_2 NPs [122]. It was shown in a previous study on healthy volunteers that oral administration of TiO_2 NPs led to absorption of NPs in intestine and their transportation to the bloodstream [123]. Moreover, analysis of post-mortem human livers and spleens revealed the presence of

TiO_2 NPs, which indicates that NPs have the potential to accumulate in the human body [124].

1.4.3 Putative toxicity of TiO_2 NPs

TiO_2 is a poorly soluble material, and therefore its possible toxic effects are related to the size and/or other properties of the particles than to the release of metal ions [125]. Recent studies have shown that NPs' toxicity is determined by their physicochemical characteristics, such as size, shape, surface area, surface charge, polydispersity index, agglomeration rate and by the biological environment that NPs come in contact with [17, 126, 127]. Althought the results of some studies are contradictory, it is considered that TiO_2 NPs with the size between 10 and 30 nm, anatase cristalline structure, spherical shape, higher surface charge and less tendency for agglomeration exhibit higher cytotoxicity [128-131].

The mechanism of NPs' toxicity is not completely understood. However, the hypothesis that has gained support is that NPs' toxicity is associated with the production of reactive oxidative species (ROS) and consequent oxidative stress, which may lead to inflammation, fibrosis, genotoxicity, and carcinogenesis [3, 17, 132]. The possible genotoxicity mechanisms include direct interaction of NPs with genetic material or most commonly reported, indirect DNA damage through NP-mediated ROS production [6].

Based on the evidence that micronized TiO_2 particles (1.5 - 1.7 μm) can cause lung cancer in rats, they were classified as possibly cancerogenic for humans [133]. However, in 2019, an expert panel of scientists and experts in inhalation risk assessment questioned the relevance of rat studies for human carcinogenicity assessment [134]. First, the rats who developed cancer underwent chronic overload of TiO_2, which is not relevant for human exposure and, second, the results from rat studies were not reproduced in other species (hamsters and mice) [135, 136]. Most of the panel experts agreed that rats are more sensitive in their lung response to TiO_2 NPs than other species and humans [137]. Recently, it was concluded that TiO_2 NPs have low toxicity and nano-TiO_2 classification as "possible carcinogen" should be reassessed [138]. As

a result, nano-TiO_2 is no longer classified as carcinogenic by inhalation in powder form [139]. In contrast, the European Commission has recently prohibited the usage of TiO_2 NPs in food because they were found to alter gut microbiota and have exhibited carcinogenic potential in rats [11, 140-142]. The uncertainty regarding the use of these NMs in food is associated with the difficulty to establish the safety dose for oral intake of TiO_2 NMs [143]. The abovementioned decision was met with criticism by different stakeholders and experts. In a recent review of genotoxicity studies, Kirkland et al. (2022) concluded that existing evidence does not support the direct damaging mechanism of TiO_2 NPs and that these damages are most likely secondary to physiological stress [5]. It was also stated that to definitely exclude the mutagenic effect of TiO_2 NPs there is a need for more robust *in vitro* and *in vivo* studies. This illustrates the lack of consensus on the safety of widely used NMs, and thus there is a need for toxicological studies and new methodologies to provide reliable evidence relevant for human exposure and to support decision making [143].

Cellular uptake of NPs

Cytotoxic effects of NPs are mostly associated with their presence inside the cells. NPs can enter cells by passive processes, such as diffusion. However, the most common way of NPs uptake is via active processes, such as endocytosis [144, 145]. Endocytosis includes the formation of cell membrane vesicles, which can take up different substances from the extracellular to the intracellular environment. Endocytosis has two main mechanisms – phagocytosis (cellular uptake of particles) and pinocytosis (cellular uptake of fluids and dissolved solutes) [144].

It is important to note that when NPs come in contact with biological fluids (or cell culture medium) they attract surrounding biomolecules (proteins, lipids, *etc.*) which form a corona around the particle. Presence of a protein corona together with other factors, such as surface chemistry, size, shape, surface area, agglomeration and stability in biological conditions influence the mechanism of uptake, transportation and fate of NPs in cells and tissues [145].

1.4.4 Oral mucosa

Oral mucosa is the lining tissue of oral cavity. Depending on the function, oral mucosa is divided into three types: (1) lining mucosa, covering mobile structures, such as floor of the mouth, cheeks and lips; (2) masticatory mucosa, covering alveolar bone and hard palate; and (3) specialized mucosa, located on the dorsum of the tongue [146].

Oral mucosa consists of stratified squamous epithelium (SSE), underlying connective tissue (lamina propria), and a basement membrane that separates the first two [147]. Stratification of epithelium is achieved by proliferation and sequential differentiation of the cells. As proliferating cells of the basal layer mature and undergo differentiation, they are pushed towards the epithelial surface, being ultimately desquamated and lost from the surface of the epithelium. Depending on the type of terminal differentiation, SSE can be keratinized (masticatory mucosa) and non-keratinized (lining mucosa) [147].

The barrier function of SSE is maintained by its structural properties and specific cell-to-cell adhesions [148]. In addition, a delicate balance between cell proliferation in the basal cell layer and the elimination of terminally differentiated cells from the surface by desquamation (through a specialized form of programmed cell death) plays a crucial role in the barrier function of all stratified epithelia, including the oral epithelium. Therefore, any factors influencing proliferation/desquamation processes will have an impact on epithelial barrier function, and vice versa, anything that affects the barrier function will likewise influence these processes, including the time required for one cell to move from the basal cell layer to the superficial cell layer and desquamation (the turnover). An important role in the maintenance of epithelial homeostasis is also played by E-cadherin [149]. It is a protein on the surface of cell membrane that is responsible for cell-to-cell adhesion. Lack of E-cadherin in intestinal epithelium has been coupled to increased permeability to external pathogens and development of chronic inflammatory conditions, including Chron's disease, indicating a link between functionality of E-cadherin as an adhesion molecule and tissue integrity [149]. Previous studies on 3D oral mucosa models have used E-cadherin as a marker of epithelial integrity [150, 151].

1.4.5 *In vitro* models

Studies on toxic effects of NPs show poor correlation between the results of *in vitro* and *in vivo* tests [152]. Conventional *in vitro* 2D cell culture studies can provide important information about interactions between NPs and cells, allowing a mechanistic exploration of such interactions [153]. However, they fail to account for complex multilevel structures of epithelial barriers in interaction with the subjacent connective tissue and for physiological processes, such as epithelial turnover and desquamation (shedding of upper layers of epithelium). They also lack blood, lymphatic and immune components which are essential parts of any tissue. In addition, the presence of saliva, in particular its quantity, composition, and pH level could be critical for NPs' toxicity [154]. The abovementioned limitations of 2D cell cultures could be partly overcome by employing 3D cell culture models.

The main advantage of 3D cell culture models over 2D cell cultures is that they reproduce the differentiated structure and function of native tissues [155]. Furthermore, the fact that 3D models are cultured in an air-liquid interface allows the exposure to be closer to real life conditions, compared to 2D cell cultures, where the exposure is challenged by the presence of cell culture medium [155]. 3D cell culture models have a variety of applications in cancer research, drug studies, investigation of cell physiology, tissue engineering and toxicological studies [156, 157]. Several 3D oral mucosa models have been developed for various purposes [158-163]. A few 3D oral models have been commercialized and are available on the market – SkinEthic™ Human Oral Epithelium and SkinEthic™ Human Gingival Epithelium (both from Episkin, Lyon, France) and EpiOral™ and EpiGingival™ tissues (both from MatTek Corporation, Ashland, MA, USA).

Although the use of 3D oral mucosa models is increasing, their application for nanotoxicity screening is still very limited [164, 165]. Alternatively, *ex vivo* models of excised porcine buccal mucosa have been used as they exhibit the same permeability as human buccal mucosa [166-169].

1.5 Rationale for the present study

The issue of nanosafety is of high relevance since novel NMs can pose risks to human health and the environment [2]. A number of EU projects, *i.e.*, RiskGONE, NANORIGO, Gov4Nano, have been launched, aiming to develop a science-based risk governance framework for NMs and to facilitate transparent risk communication between all stakeholders and the civil society [170]. To date, there is not enough evidence on the safety of NMs, which can promote fluctuation in public opinion. Uncertainty regarding nanosafety might compromise public trust in regulatory authorities and lead to opposition to nanotechnology [171]. In this respect, the evaluation of public attitudes and risk perceptions regarding the use of NMs are important goals [172, 173]. Research in this field has not been done in the general Norwegian population, or among dental health care workers.

To date, our understanding of interaction of NMs with oral cells and tissues is insufficient [119]. Toxicological data that is reliable and relevant to real-life exposure is needed to give a strong basis for decision-making by regulatory authorities. Clearly, there is a need for more *in vitro* studies employing NAMs, including methods that are less prone to interferences from NMs, as well as biomimetic experimental models, in order to assess the potential toxicological effects of NMs [23].

2. Aims

2.1.1 Overall aim

The overall aim of the present study was to address the issue of nanosafety in dentistry from two different perspectives: (1) assessment of risk/benefit perceptions and intention to use NMs by dental professionals and (2) evaluation of *in vitro* cytotoxic effects of TiO_2 NPs related to oral and lung exposure using NAMs.

2.1.2 Specific aims

I. To assess whether socio-demographic factors, familiarity with nanotechnology and social trust, are associated with dental health care workers' perceived risks and benefits of the use of NMs in dentistry and whether those associations vary according to the professional status (Study I).

II. To predict dental health care workers' intention to use NMs in the future and to explore whether the augmented TPB model operates equivalently across the professional groups of dentists and dental hygienists (Study II).

III. To assess the effects of spherical and spindle-shaped TiO_2 NPs on the viability and proliferation of primary human normal oral fibroblasts, lung cancer epithelial cells and on 3D RNHBM models. The integrity of 3D RNHBM epithelium was also evaluated (Study III).

3. Methods

3.1 Survey (Study I and II)

3.1.1 Recruitment of participants and study design

The data presented in Study I and II stem from one cross-sectional national study conducted in 2017 in Norway. A census of dentists and dental hygienists (1792 employees in total, 1255 dentists and 537 dental hygienists) employed in the public dental health care services (PDHS) in 18 (out of 19) counties in Norway were invited to participate in the survey during the period from March until May 2017. The chief dentist of 1 county didn't give permission to the survey due to the conduction of another survey in the same period which resulted in the withdrawal of 163 employees. Dental health care workers' e-mail addresses were obtained from the chief dentists of participating counties. Main invitation to participate in the survey was followed by three reminders in order to increase the response rate. In addition, a lottery was drawn with two gift cards as an incentive.

3.1.2 Questionnaire

Data was collected by the use of self-administered, structured, electronic questionnaires. The questionnaire was based on a questionnaire from the previous study among dental students in Norway and Romania [96], relevant literature [73, 90, 94, 174-176] and guidelines for TPB questionnaires [50, 177]. According to the guidelines, the behavior of interest should be defined in terms of four elements – its target, the action involved, the context in which it occurs and the time frame [50, 52]. Each of the four elements could be defined at different level of specificity. According to the key principle of the TPB (the principle of compatibility), each construct of TPB must correspond to the behavior in terms of specificity of all four elements. In the present study, the behavior of interest was defined as the "use of NMs in dentistry in the future". Consequently, attitudes, subjective norms and PBC were assessed at the same specificity level as the behavior of interest.

The questionnaire was constructed in Norwegian and comprised questions about socio-demographics (6 items), reading literacy (2 items), knowledge about NMs (12 items), attitudes towards NMs (9 items), intention to use (4 items) and past experience with NMs (2 items), perceived behavioral control (5 items), subjective norms (6 items), perceived risks related to NMs (6 items), perceived benefits related to NMs (6 items), being worried about NMs (2 items), safeness to use NMs (1 items), trust in stakeholders (2 items) and interest in information about NMs (3 items) (Appendix I).

The questionnaire was pilot tested among dental health care workers (n = 7) in one public dental clinic in Bergen in order to assess whether the formulation of the questions was easily understandable. After the pilot study, minor corrections were performed to improve the clarity of several items of the questionnaire. The final version was approved by Norwegian Centre for Research Data (NSD) which was also responsible for distribution of questionnaires by email, data collection and anonymization of personal data. Estimated time to complete the questionnaire was 15-20 min. The employees got permission from county chief dentists to fill out the questionnaire during their working hours.

In **Study I,** covariates of perceived risks and benefits associated with the use of NMs in dentistry were identified. Dependent variables included perceived risks and benefits. Each was measured as an additive index of six items. Independent variables included socio-demographic factors, knowledge about NMs, amount of received information about NMs, past experience, reading literacy, being worried, safeness to use NMs and trust in stakeholders.

In **study II,** the original constructs of TPB (intention, attitudes, perceived behavioral control and subjective norms) were based on several single items. In addition, the model was augmented with two external variables – risk perception and past behavior. Risk perception was a summative score of six items, while past behavior was measured by one item.

3.2 *In vitro* toxicity study (Study III)

3.2.1 Materials

Table 2. Overview of the materials used in *in vitro* toxicity study.

Name	Supplier	Catalog number
A549 Cell line	ATCC	CCL-185
Normal Oral Fibroblasts	-	-
Dulbecco's Modified Eagle's Medium (DMEM)	Sigma	D6046
DMEM/Nutrient Mixture F-12 Ham	Sigma	D8437
Keratinocyte Serum Free Medium (KSFM)	Gibco	17005042
Insulin-Transferrin	Gibco	41400045
Fetal Bovine Serum (FBS)	Invitrogen	10270106
Antibiotic-antimycotic	Gibco	15240062
Trypsin EDTA	Sigma	T4174
Ascorbic Acid	Sigma	A7631
Hydrocortisone	Sigma	H0888
Epidermal Growth Factor (EGF)	Sigma	E4269
Trypan Blue 0.4%	Invitrogen	T10282
Xylene		
Ethanol		
Target Retrieval Solution pH6 (10x)	Agilent Dako	S2369842
Target Retrieval Solution pH9 (10x)	Agilent Dako	S2367842
Tris-buffered Saline with 0.1% Tween® 20 detergent (TBST)	Agilent Dako	
PAP Pen	Agilent Dako	S2002
Peroxidase-Blocking Solution	Agilent Dako	S2023862
Protein Block	Agilent Dako	X0909302
EnVision FLEX Antibody diluent	Agilent Dako	K8006212
EnVision FLEX+ Mouse Linker	Agilent Dako	K8002212
Goat serum	Agilent Dako	X0907108
EnVision FLEX/HRP	Agilent Dako	K8000
EnVision+ Single Reagent (HRP. Rabbit) (secondary ab for Clcasp3)	Agilent Dako	K4011
EnVision FLEX DAB+ Chromogen	Agilent Dako	DM827
EnVision FLEX Substrate Buffer	Agilent Dako	GV825
Hematoxilin	Agilent Dako	S3301
Monoclonal Mouse Anti-Human Ki-67 Antigen	Agilent Dako	M7240012
Monoclonal Mouse Anti-Human E-Cadherin	Agilent Dako	M3612012
Monoclonal Rabbit Cleaved Caspase-3 (Asp175) (5A1E)	Cell Signaling Technology	9664S
Pertex Mounting Medium	HistoLab	00811

3.2.2 Nanoparticles

TiO_2 NPs were used in this study as they are included in dental materials, medical equipment, personal care products, such as toothpaste, and, until recently, food. Spherical NPs with a primary particle size of 40 nm and spindle-shaped NPs with a primary particle size of 10 x 40 nm were purchased from American Elements ®, Los Angeles, CA, USA and Nanostructured and Amorphous Materials Inc., Garland, TX, USA, respectively. Both types of NPs had rutile crystalline structure.

Exposure suspension

Stock solutions of NPs in distilled water at a concentration of 5g/l were prepared. In order to minimize agglomeration and provide a homogeneous NPs' dispersion, sonication was performed by using an ultrasonicator (VCX130, Vibra-Cell, 130W, Sonics & Materials Inc., USA) with 12.8 mm probe with a replaceable tip for 1 min at 70% duty. The use of a sonication probe is considered a standardized method for preparation of TiO_2 NPs' dispersions [178, 179]. During sonication, the bottle with stock solution was immersed in a beaker with iced water to avoid overheating of solution which can result in NPs agglomeration. Immediately after sonication, exposure suspensions were prepared by adding the necessary amount of stock solution to complete cell culture medium. The final concentrations for exposure of 3D RNHBM models were 5, 20 and 2000 μg/ml, based on which corresponding concentrations for exposure of cells in monolayers were calculated, so that the concentrations of NPs per surface area would be equal in both 2D and 3D studies. The matching concentrations for 2D cell cultures were 0.2, 1 and 100 μg/ml. Two additional concentrations of 10 and 80 μg/ml were added for 2D bioimpedance-based testing, as the e-plates employed allowed to test up to 5 concentrations. Exposure was performed immediately after the suspensions were ready.

Physicochemical characteristics of TiO_2 NPs in suspension

NPs size (hydrodynamic diameter) and surface charge (Zeta-potential) in suspension were measured with a Zetasizer Nano ZSP instrument (Malvern Instruments, Malvern, UK), utilizing dynamic and electrophoretic light scattering (DLS and ELS),

respectively. Both methods are based on the measurement of scattered light from the Brownian motion of the particles in liquid media. Due to their simplicity and reproducibility, the techniques are routinely used for NPs' characterization. Hydrodynamic diameter reflects the size of the particle in liquid media, while Zeta-potential reflects the stability of colloidal solution [180, 181]. As suggested earlier, to correlate properties of NPs to their toxicity potential, DLS and ELS measurements were performed right before and 24 h after the exposure (end of exposure) [180].

3.2.3 Cell culture

Considering that inhalation and ingestion are the most common routes of general exposure to NPs, lung cancer epithelial cells (A549) and primary normal human oral fibroblasts were employed in the 2D cell cultures. A549 is a commonly used cell line in nanotoxicity studies addressing lung exposure [182, 183]. The cells were purchased from the American Type Culture Collection (ATCC, CCL-185, LGC Standards GmbH, Germany). Primary oral fibroblasts were isolated from oral mucosa obtained during third molar extraction from healthy volunteers after informed consent. Oral fibroblasts were then isolated from the connective tissue of the buccal mucosa by using an explant culture method and propagated in DMEM with 10% FBS and 1% antibiotic/antimycotic [159]. Both A549 and oral fibroblasts were used in the experiments if their viability was above 90% and passage number was below 15. Cells were grown in a humidified CO_2 atmosphere and regular mycoplasma testing with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza Walkersville, Inc.) was performed.

3.2.4 Reconstructed Normal Human Buccal Mucosa Model

A 3D coculture model developed at the Oral Pathology section (Faculty of Medicine, University of Bergen) by Costea et al. (2003) [159] resembles native tissue in terms of structure and homeostasis and is therefore more relevant for human oral exposure to TiO_2 NPs, compared to 2D cell cultures. The 3D RNHBM models in this study were constructed from keratinocytes and fibroblasts isolated from buccal mucosa of 5 healthy volunteers who underwent extraction of the impacted third molar. (Figure 3).

All volunteers read and signed informed consent before tissue collection. Detailed description of the procedure can be found elsewhere [165].

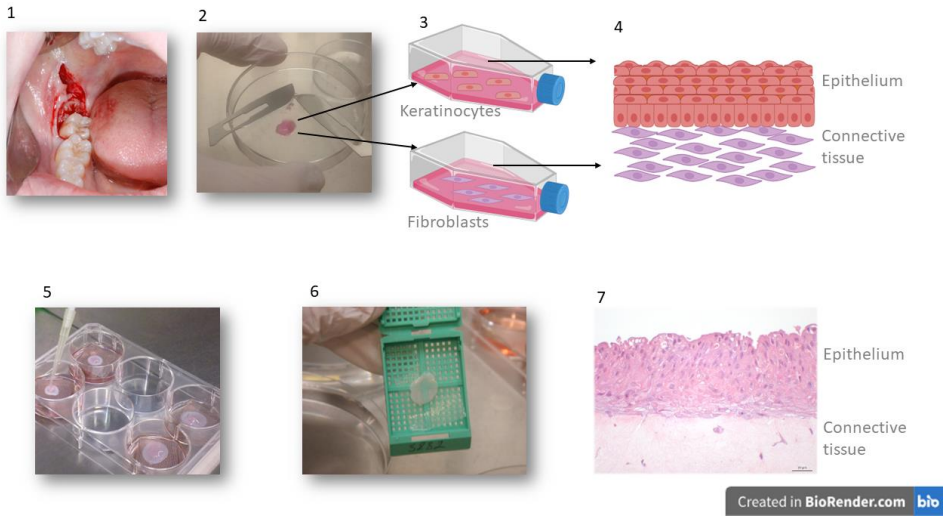

Figure 3. Illustration of construction and exposure of 3D RNHBM model. 1) Site of biopsy collection (*Image 1 courtesy S. Kvalheim* [150]) 2) Separation of epithelial and connective tissues 3) Cell isolation and propagation 4) The main compartments of the oral mucosa reconstructed in the 3D model 5) TiO_2 NPs' exposure of 3D models cultured in air-liquid interface 6) Harvesting of the 3D models 7) Histology of a 3D RNHBM model (H&E staining) under the light microscope (Scale bar 20µm)

3.2.5 Impedance-based monitoring to measure cell proliferation and viability

In our study, an xCELLigence real-time cell analysis system (ACEA Biosciences, CA, USA) and E-plates with gold-plated electrodes on the bottom of each well were used. An alternating electrical current was sent through the electrodes every 15 min and the response (electrical impedance) was measured. Viable cells attached to the electrodes impede the electrical current. The results are reported by the xCELLigence system software as Cell Index (CI), which is a unitless parameter:

CI = (impedance at timepoint n – impedance in the absence of the cells) / nominal impedance value

When the cells are proliferating, the CI is increasing, when the cells are detaching from the bottom and/or the cell membrane becomes permeable, the CI is decreasing, which is indicative of cell death [184, 185].

The A549 cells and primary oral fibroblasts were seeded in 16-well e-plates at a density of 5.000 cells/well (25.000 cells/cm^2) and 10.000 cells/well (50.000 cells/cm^2), respectively. Since the oral fibroblasts proliferate slower than A549 cells, the initial seeding density for oral fibroblasts was higher than for A549 cells in order to achieve a similar number of cells at the time of exposure to TiO_2 NPs, i.e., 24 h after cell seeding.

3.2.6 Immunohistochemistry

The effects of NPs on 3D RNHBM models in terms of proliferation, apoptosis, and tissue integrity were assessed with the help of immunohistochemistry. Nuclear protein **Ki-67** is a proliferation marker as it is highly expressed in the nucleus of cycling cells (G1, S, G2 and M-phases), but absent in quiescence cells (G0-phase) [186]. Its presence in reconstructed epithelium was detected by a monoclonal Ki-67 antibody (1:100, Agilent Dako). **Cleaved caspase-3** is an activated form of caspase 3, which in its inactive form is typically found in the cytoplasm of cells. During apoptosis, caspase 3 is activated (cleaved) and translocated to nucleus, where it cleaves DNA, leading to DNA fragmentation, chromatin condensation and nuclear disruption [187, 188]. Its presence was detected by monoclonal cleaved caspase-3 antibody (1:200, Cell Signaling Technology). **E-cadherin**, also known as epithelial cadherin, is a protein that belongs to the cadherin family of cell adhesion molecules, and it is expressed in most normal epithelial tissues [189]. It is localized on the cell membrane of epithelial cells where it forms calcium-dependent homophilic interactions with E-cadherin molecules on adjacent cells. This interaction helps to create stable adherent junctions, which are crucial for cell-to-cell adhesion and the maintenance of structural tissue integrity in epithelial tissues such as skin, oral mucosa and the lining of digestive tract [189]. As it is involved in the control of cell adhesion and tissue organization, loss of E-cadherin is pathological, and is often associated with cancer [190]. In our study, a monoclonal E-cadherin antibody (1:25, Agilent Dako) was used to detect this molecule.

3.2.7 Image analysis and quantification

After immunohistochemistry, the sections of RNHBM were covered with cover glass using Pertex Mounting Medium (Histolab). The slides were then scanned using Aperio Scanscope® CS Slide Scanner (40 x magnification) and the images were analyzed with the help of QuPath software for digital pathology image analysis [191]. Proliferating (Ki-67 positive) and apoptotic (cleaved caspase-3 positive) cells were counted in basal and suprabasal layers of epithelium. The basal layer was defined as the first cell layer at the bottom of epithelium, while the rest of epithelium was defined as the suprabasal layer (Figure 4). Tissue integrity (expression of E-cadherin) was assessed across the whole epithelium. The ratio of proliferating, apoptotic cells and cells expressing E-cadherin were calculated as percentage of positively stained cells out of all cells detected.

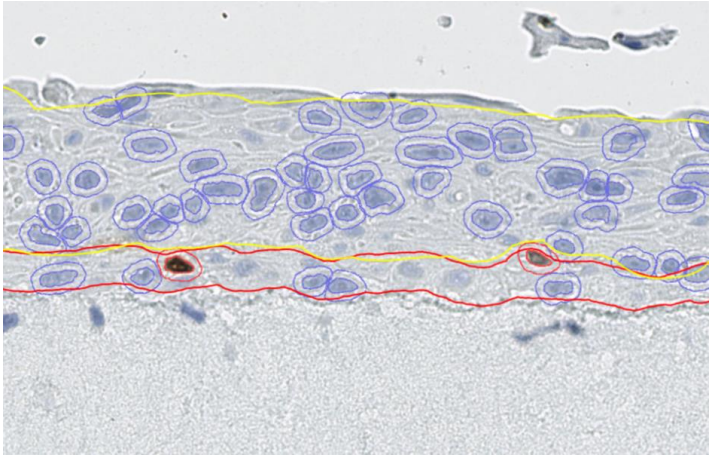

Figure 4. Illustration of cells quantification on a cross-section of 3D RNHBM model. For the marker of proliferation (Ki-67) and apoptosis (cleaved caspase-3) the cells were counted in basal (red outline) and suprabasal (yellow outline) layers of epithelium. Positively stained cells are outlined with red circles, negatively stained cells are outlined with blue circles.

3.2.8 Transmission electron microscopy

In our study a JEM-2100 microscope (JOEL, Japan) was used to examine the morphology of NPs suspended in deionized water at a concentration of 100 µg/ml and to assess NPs' internalization into cells exposed to both types of NPs at 100 µg/ml.

3.3 Statistical analysis

Table 3. Overview of statistical methods and analyses used in the thesis.

Statistical test	**Study I**	**Study II**	**Study III (*in vitro*)**
Descriptives	x	x	x
Cross tabulation	x		
Binary logistic regression	x		
Confidence interval	x	x	
Nagelkerke's R^2	x		
Confirmatory factor analysis (CFA)		x	
Structural equation modelling (SEM) with full information maximum likelihood (FIML)		x	
Chi-square test X^2		x	
Comparative fit index (CFI)		x	
Root mean square error of approximation (RMSEA)		x	
Standardized root mean square residual (SRMR)		x	
Cronbach's alpha		x	
General linear model			x
Mixed effects model			x
Intraclass correlation coefficient			x

All analyses were performed using SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) and Stata 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.). Articles I and II operate with one unit of analysis – the unit of individual which is also a unit of observation. In study III, the 2D cell and 3D model are the unit of analysis and clustering of 3D models within individuals have been accounted for.

In **Study I** descriptive statistics were performed to calculate the frequency distribution of independent and dependent variables. Further, cross tabulation with chi square test was conducted to examine the relation between each independent and dependent variable. Finally, multiple variable binary logistic regression analysis with odds ratios

and 95% CI were used to estimate the independent effect of all predictor variables on the outcomes.

In **study II** descriptive statistics were performed to calculate the frequency distribution of socio-demographic variables. Further, structural equation modelling (SEM) was used to investigate how well the hypothesized augmented TPB model fits the data and to test the direct and indirect relationships between the constructs in the model. A two-step modelling approach was used. In the first step, adequacy of the measurement model was tested with the help of confirmatory factor analysis (CFA). Potential sources of misfit were identified with modification indices, which provided a basis for re-specification of the model until the adequate fit was achieved. In the second step, SEM was performed to examine how well the hypothesized augmented TPB model fits the data and to estimate direct, indirect and total effects of relationships in the model. To measure how well the model fit the data, the following statistical parameters were used – Chi-square test (X^2), comparative fit index (CFI), root mean square error of approximation (RMSEA) and standardized root mean square residual (SRMR) (Kline, 2011). A good fit of the model is indicated by statistically insignificant Chi-square test (X^2) with $p > 0.05$. An acceptable and good fit is indicated by values of CFI > 0.90 and > 0.95, RMSEA < 0.08 and < 0.06 and SRMR < 0.08 and < 0.05, respectively (Hu and Bentler, 1999). The maximum likelihood estimator with robust standard errors (MLR) was applied to account for non-normally distributed data. Missing data was handled by the full information maximum likelihood (FIML) which is considered superior to standard ad hoc routines such as mean replacement and listwise or pairwise deletion [192]. To test whether the model was invariant across the two professional groups of participants, multigroup analyses were performed in both steps (CFA and SEM). In the first step (CFA) configural and metric invariance was tested. Configural invariance means that the pattern of item loadings on the latent factor is the same across the groups [193]. Configural invariance of the final measurement model was tested by fitting the final measurement model across dentist and dental hygienists. If the fit of the model was acceptable (based on abovementioned fit indices), configural invariance was supported. After that, metric invariance was tested. Metric invariance means that each

item loads on its factor to a similar degree across the groups [193]. It was tested by constraining factor loadings in both groups and comparing the constrained model with the baseline model (configural invariance model) in which factor loadings were free to vary. Metric invariance was supported if Chi-square delta was non-significant and CFI delta was less than 0.002 [194]. Last, invariance of regression paths was tested by comparing a structural model where both factor loadings and regression paths were constrained across the groups to a baseline structural model where factor loadings were constrained, and regression paths were free to vary. The invariance of predictive paths was supported if Chi-square delta was insignificant and CFI delta less was than 0.002.

In **Study III** data were presented as mean ± SE. Data from 2D cell cultures was expressed as normalized cell index (CI) and analyzed with the use of general linear models. Data from 3D buccal mucosa models was expressed as percentage of positively stained cells and analyzed with mixed effect model. This model accounted for possible correlation of the samples within the patient and estimated intraclass correlation coefficients (ICC). A P-value of < 0.05 was considered statistically significant.

3.4 Ethical approval

The ethical considerations were in accordance with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects [195] and the Act on Medical and Health Research [196].

Regional Committee for Medical and Health Research in Norway has approved the study on human cells and 3D RNHBM models (2013/1492/REK Vest). Informed consent was collected from the patients who agreed to participate in the study.

The survey was approved by the Norwegian Centre for Research Data (51053/3/AMS). In addition, the survey was approved by chief county dentists. Informed consent was provided electronically together with the invitation to the study. By completing the online questionnaire participants were giving their consent to participate.

4. Results

4.1 Study I

Use of nanomaterials in dentistry: covariates of risk and benefit perceptions among dentists and dental hygienists in Norway

Aim: To assess whether socio-demographic factors, familiarity with nanotechnology and social trust, are associated with dental health care workers' perceived risks and benefits of the use of nanomaterials in dentistry and whether those associations vary according to the professional status.

Out of 1792 dental health care workers invited to participate in the survey 851 completed electronic questionnaires (47.5% response rate). Of the respondents 18.6% were males, 39.5% were below 36 years old, 71.4% were dentists. Distribution of dental health care workers by professional status and genders in the survey corresponds to that in PDHS in Norway (Table 4). Moreover, the age distribution of the participants was also in accordance with that of dental professionals employed in PDHS (Table 5).

Table 4. Distribution of dental health care workers employed in PDHS in Norway and survey participants by professional status and gender.

	PDHS employees*, % (n)	**Survey participants, % (n)**
Dentists	**71.2 (1391)**	**71.4 (570)**
Male	24.6 (342)	25.6 (139)
Female	75.4 (1049)	74.4 (404)
Dental hygienists	**28.8 (564)**	**28.6 (228)**
Male	1.9 (11)	1.4 (3)
Female	98.1 (553)	98.6 (218)
Total	**100 (1955)**	**100 (798)**

*According to Statistical Central Agency in 19 counties in 2017

Table 5. Distribution of dental health care workers employed in PDHS in Norway and survey participants by age group.

	PDHS employees*, % (n)	Survey participants, % (n)
Dentists		
Below 39	55.9 (775)	58.2 (309)
40-59	32.3 (448)	31.6 (168)
Above 60	11.8 (164)	10.2 (54)
Total	**100 (1387)**	**100 (531)**
Dental hygienists		
Below 39	44.0 (248)	34.4 (75)
40-59	41.7 (235)	51.8 (113)
Above 60	14.4 (81)	13.8 (30)
Total	**100 (564)**	**100 (218)**

*According to Statistical Central Agency in 19 counties in 2017

High perceptions of risks and benefits were reported more often by dentists than dental hygienists – 69.1% dentists versus 50.6 dental hygienists ($p < 0.01$) for risks and 77.3% dentists versus 44.0% dental hygienists ($p < 0.01$) for benefits. Almost three times more dentists reported to have moderate or more knowledge about NMs compared to dental hygienists (60.4% dentists versus 23.3% dental hygienists, $p < 0.01$). Correct knowledge (based on test) was demonstrated by 62.2% dentists and 38.0% dental hygienists ($p < 0.01$). Previous use of dental NMs was confirmed by 63.7% dentists and 28.7% dental hygienists ($p < 0.01$).

Multiple variable logistic regression model revealed that having previous experience with NMs (OR = 2.2, 95% CI 1.3-3.7), having high trust in stakeholders (OR = 2.6, 95% CI 1.4-4.6), being a dentist (OR = 3.6, 95% CI 2.1-6.2) and feeling safe (OR = 6.6, 95% CI 3.1-14.2) increased the odds of high benefits perceptions related to the use of NMs. The final model explained 42.3% of the variance of benefit perceptions. Being a dentist (OR = 1.9, 95% CI 1.1-3.3), having high trust in stakeholders (OR = 2.1, 95% CI 1.3-3.4), having moderate and much correct knowledge about NMs (OR = 2.3, 95% CI 1.5-3.5) and being worried about increasing use of NMs (OR = 9.3, 95% CI 4.5-19.3) increased the likelihood for risk perceptions. The final model explained 26.6% of the variance of risk perceptions.

4.2 Study II

Predicting intention of Norwegian dental health-care workers to use nanomaterials: An application of the augmented theory of planned behavior

Aims: To predict dental health care workers' intention to use NMs in the future and to explore whether the augmented TPB model operates equivalently across the professional groups of dentists and dental hygienists

Cronbach's alfa values of the constructs included in TPB model (intention, attitudes, subjective norms, perceived behavioral control, and risk perceptions) varied from 0.8 for perceived behavioral control to 0.93 for attitudes and intention indicating high internal consistency. CFA revealed that the hypothesized four-factor model had an acceptable fit (Table 6, Model 1). With the help of modification indices, the model fit was improved by adding residual correlations to the model (Table 6, Model 2 - 4). The final measurement model achieved a god fit (Table 6, Model 4). Configural invariance of the measurement model was supported as the model had acceptable fit when applied across the two professional groups (X^2 = 522.9 (DF = 200), $p < 0.001$, CFI = 0.947, RMSEA = 0.063 (CI = 0.057-0.070), SRMR = 0.048) for dentists and (X^2 = 285.7 (DF = 200), $p < 0.001$, CFI = 0.946, RMSEA = 0.058 (CI = 0.42-0.73), SRMR = 0.062) for dental hygienists. In addition, metric invariance was supported as indicated by non-significant chi-square change (ΔX^2 = 0.655 (DF = 400-418, $p > 0.05$)) and CFI change less than 0.002 (ΔCFI = 0.000), implying the constructs were interpreted in the same way across the two professional groups.

Table 6. Overall goodness of fit indices for the TPB measurement models (Model 1-4) and full structural model (Model 5)

Fit indices	Model 1	Model 2	Model3	Model 4	Model 5
X^2	782.3	680.6	612.8	555.9	665.5
DF	203, P<0.000	202, P<0.000	201, P<0.000	200, P<0.000	236, P<0.000
CFI	0.926	0.940	0.948	0.956	0.946
RMSEA	0.075	0.068	0.063	0.058	0.063
90%CI RMSEA	0.069-0.080	0.062-0.074	0.057-0.069	0.053-0.064	0.058-0.069
SRMR	0.049	0.048	0.045	0.042	0.045

X^2: Chi-square test; DF: degrees of freedom; CFI: comparative fit index; RMSEA: root mean square error of approximation; CI: confidence interval; SRMR: standardized root mean square residual

The full structural model had a good fit (Table 6, Model 5). Multi-group analysis revealed that the fit of the model where regression paths were constrained was not significantly worse than the fit of the model where regression paths were free to vary ($\Delta X^2 = 0.32$ (DF = 490-501, $p > 0.05$), $\Delta CFI = 0.000$), which supports the invariance of regression paths across the two professional groups.

According to SEM, the strongest predictors of intention to use dental NMs were attitudes ($\beta = 0.53$, $p < 0.001$) and perceived behavioral control ($\beta = 0.24$, $p < 0.001$), followed by past behavior ($\beta = 0.15$, $p < 0.001$) and subjective norms ($\beta = 0.11$, $p < 0.05$) (Figure 5). This implies that those dental health care workers' who had favorable attitudes towards the use of NMs, who thought that it would be easy to use NMs, who had used them before and who thought that their colleagues or chief dentists approve their use of NMs had higher intention to use such materials. Although risk perception didn't have any direct effect on intention ($\beta = 0.00$, ns), its indirect effect through attitudes, PBC and SN was significant and in negative direction ($\beta = - 0.21$, $p < 0.001$), which implies that high risk perception is associated with low intention to use NMs. According to R-squared, the augmented TPB model (including intention, attitudes, subjective norms, perceived behavioral control, past behavior, risk perceptions) explained 74.5% of the variance of intention to use NMs compared to original TPB model (without past behavior and risk perceptions) which explained 71.8% of the variance.

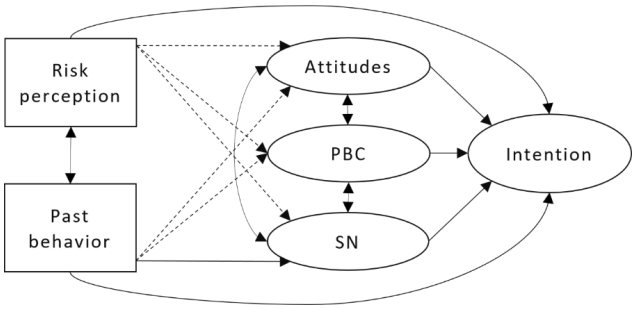

Figure 5. The hypothesized augmented TPB model including four latent (intention, attitudes, PBC and subjective norms) and two observed (risk perception and past behavior). Solid and dashed lines represent direct and non-direct effects, respectively.

4.3 Study III

Effect of TiO_2 nanoparticles on cell proliferation and viability: An in vitro study on 2D and 3D biological models

Aims: To assess the effects of spherical and spindle-shaped TiO_2 NPs on the viability and proliferation of primary human normal oral fibroblasts, lung cancer epithelial cells and on 3D RNHBM models. The integrity of 3D RNHBM models was also evaluated.

TiO_2 NPs characterization

Detailed characterization of NPs in powder was performed by Allouni et al. (2015) and is reproduced here with the permission from the author and the publisher (Science Direct) [197] (Table 7). The size and morphology of the two types of NPs dispersed in deionized water (100 µg/ml) is presented in Figure 6 (A, B).

Table 7. Physicochemical characteristics of TiO_2 NPs in powder

Supplier's description	S_{BET} (m^2/g)[a]	D_{BET} [nm][b]	Crystal structure	Crystal size (nm)[c]	IEP[d]	Circularity ± SD[e]	MECD ± SD[f]
Spherical TiO_2 NPs, rutile, 40 nm (TI-OX-02-NP.050, American Elements®, USA)	38	37	92% rutile 8% anatase	Rutile: 21	-	0.79 ± 0.08	36 ± 22
Spindle-shaped TiO_2 NPs, rutile, 10×40nm (#5480MR, Nanostructured & Amorphous Materials Inc., USA)	165	Not spherical	100% rutile	Rutile: 8.5	3.10	0.29 ± 0.04	14 ± 6

[a]Specific surface area
[b]Calculated particle's diameter from Brunauer-Emmett-Teller (BET) measurements, $D_{BET} = k/ \rho \times S_{BET}$, $k = 6$ for a sphere, ρ is the density of the powder
[c]Calculated using Scherrer Equation: $D = k\lambda/ \beta \times \cos\theta$
[d]Isoelectric point from titration curve: zeta potential vs. pH in aqueous solution of 0.14 M NaCl
[e]Circularity from TEM pictures of NPs
[f]Average microscopy equivalent circle dimatere (nm) from TEM pictures of NPs

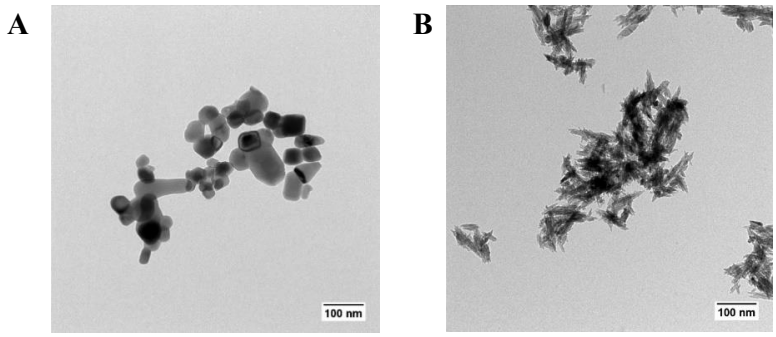

Figure 6. Transmission electron microscopy of spherical (A) and spindle-shaped (B) rutile TiO_2 NPs in deionized water at a concentration of 100 μg/ml.

The DLS of the TiO_2 NPs is stock solution (deionized water) and in complete cell culture medium revealed that spherical NPs had a larger hydrodynamic diameter (418.7 ± 4.55 nm at exposure and 335.2 ± 13.97 nm after 24h) compared to spindle-shaped (245.3 ± 1.27 nm at exposure and 225.6 ± 1.03 nm after 24 h), which means greater tendency to agglomerate for spherical NPs (Table 8). Both types of NPs had higher negative Zeta-potential values in complete cell culture medium after 24 h than at 0 h, indicating more stable solutions after 24 h. Spindle-shaped NPs were more uniform in terms of hydrodynamic diameter compared to spherical NPs, as indicated by the polydispersity index (0.137 for spindle-shaped versus 0.208 for spherical).

Table 8. Physicochemical characteristics of TiO_2 NPs in deionized water and complete culture medium.

Sample	Time	Mean HD ± SE [nm]	PDI ± SE	Zeta pot. ± SE [mV]
Spherical TiO_2 NPs				
5 g/l, deionized water	0h	381.53 ± 9.58	0.266 ± 0.006	0.763 ± 0.233
	24h	264.77 ± 1.38	0.196 ± 0.002	-0.097 ± 0.169
100 μg/ml, DMEM +10% FBS	0h	418.70 ± 4.55	0.210 ± 0.023	-6.357 ± 0.922
	24h	335.23 ± 13.97	0.208 ± 0.032	-10.141 ± 0.184
Spindle-shaped TiO_2 NPs				
5 g/l, deionized water	0h	192.30 ± 1.85	0.097 ± 0.012	-21.64 ± 0.420
	24h	187.20 ± 2.36	0.142 ± 0.006	-32.52 ± 0.442
100 μg /ml, DMEM +10% FBS	0h	245.33 ± 1.27	0.137 ± 0.008	-6.786 ± 0.919
	24h	225.64 ± 1.03	0.137 ± 0.009	-10.137 ± 0.221

HD – hydrodynamic diameter, PDI – polydispersity index, Zeta pot. – zeta potential

According to TEM, both types of NPs were internalized into oral fibroblasts and A549 cells. The NPs were mostly present in vesicles inside the cytoplasm, and occasionally inside the nucleus. Higher uptake was observed for spherical NPs than for spindle-shaped (Figure 7, C - F).

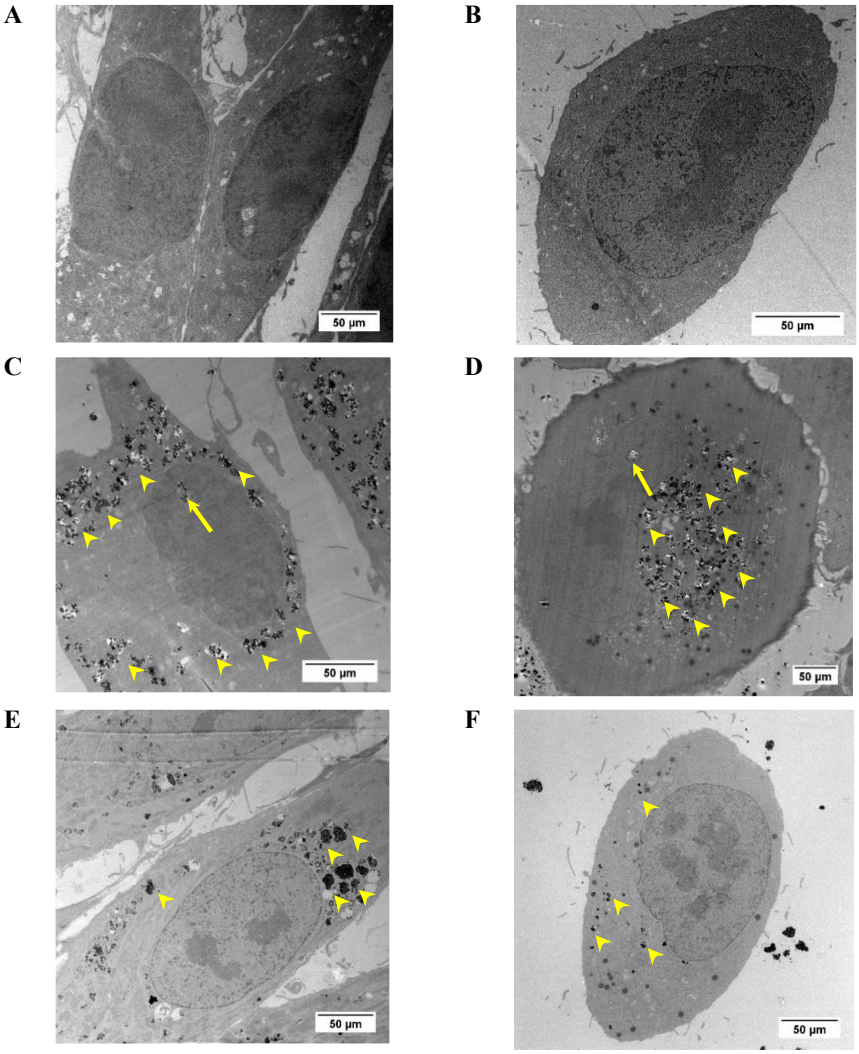

Figure 7. Transmission electron microscopy. Non-exposed oral fibroblasts (A) and A549 cells (B). Oral fibroblasts exposed to spherical (C) and spindle-shaped (E) TiO_2 NPs at a concentration of 100 µg/ml. A549 cells exposed to spherical (D) and spindle-shaped (F) TiO_2 NPs at a concentration of 100 µg/ml. TiO_2 NPs and their agglomerates can be seen in the cytoplasm, mostly in vesicles (arrowhead) and occasionally in the nucleus (arrow).

Effects of NPs on the proliferation and viability of normal and cancer cells

Impedance-based monitoring revealed a significant concentration-dependent decrease in CI for normal oral fibroblasts exposed to both types of NPs, which indicates increased cell death of exposed fibroblasts compared to controls (Figure 8, A, B). On the contrary, in lung cancer epithelial cells no significant change in CI was observed compared to controls (Figure 8, C, D). See also Figure 4 in the manuscript (Study III).

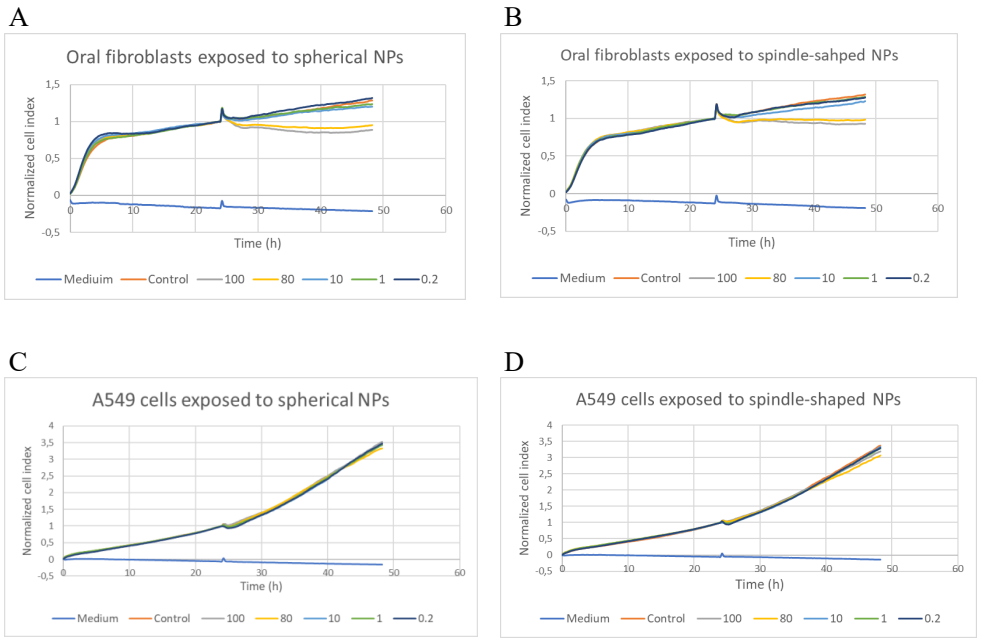

Figure 8. Normalized cell index. Oral fibroblasts exposed to spherical (A) and spindle-shape (B) TiO_2 NPs. Lung cancer epithelial cells (A549) exposed to spherical (C) and spindle-shape (D) TiO_2 NPs. Each line represents mean normalized cell index obtained from one out of three repeats.

Effects of TiO_2 NPs on cell proliferation, cell death and tissue integrity in 3D normal human oral mucosa models

Histologically, the model displayed a stratified, squamous, non-keratinized epithelium similar to that of human oral mucosa (Figure 9, A, B, C).

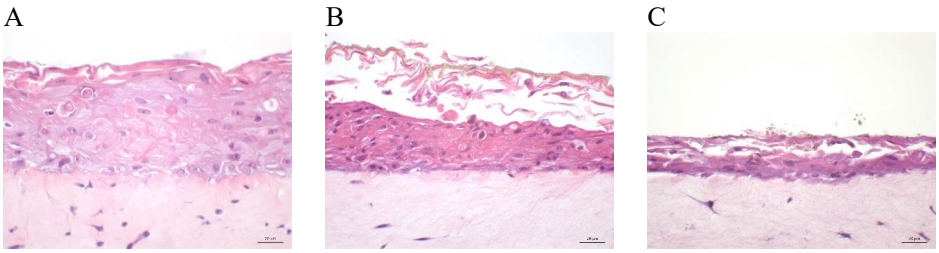

Figure 9. Light microscopy image of representative RNHBM models stained with H&E. (A) control tissue, (B) tissue exposed to 100 µg/cm^2 spherical NPs, (C) tissue exposed to100 µg/cm^2 spindle-shaped NPs. Scale bar 20 µm.

Most of the proliferating cells (as detected by Ki-67) were found in the basal layer of epithelium. There was a slight decrease in cell proliferation in the basal layer of the oral mucosa models exposed to both types of NPs at the highest concentration of 100 µg/cm^2, although it was not significantly different from the control. ICC for Ki-67 was 0.34 and 0.21 for basal and suprabasal layers, respectively.

A higher number of apoptotic cells (detected by cleaved caspase-3) was present in the basal layer of the epithelium then in supra-basal, except for tissues exposed to spherical NPs at 0.2 µg/cm^2, where more apoptotic cells were found in the supra-basal layer. Exposure to the lowest concentration (0.2 µg/cm^2) of both types of NPs was associated with decreased values of cell death in the basal layers (1.6 ± 1.3% and 2.7 ± 1.2% for spherical and spindle-shaped, respectively ($p > 0.05$)) compared to control. Increased percentages of apoptotic cells in the basal layer were found in tissues exposed to spherical NPs at 100 µg/cm^2 and spindle-shaped at 1 µg/cm^2 and 100 µg/cm^2 compared to non-exposed tissues, however the differences were not statistically significant ($p > 0.05$). ICC for cleaved caspase-3 was 0.58 and 0.15 for basal and suprabasal layers, respectively.

Expression of E-cadherin was slightly increased in exposed tissues at most of the concentrations compared to control tissues, with the highest value being seen for spindle-shaped NPs at 0.2 μg/cm^2. However, exposure to spherical NPs at 0.2 μg/cm^2 led to a slight decrease in E-cadherin expression compared to non-exposed tissues. None of the differences were statistically significant, which may indicate that the barrier function of epithelial layer was not significantly compromised. ICC for E-cadherin was 0.29.

5. Discussion

5.1 Methodological considerations

5.1.1 Study I and II

The present study has a cross-sectional design, which means that it collects data at a set point in time [198]. The advantages of this study design are that it is easy to conduct, and it allows examination of a large number of individuals and variables at a relatively low cost. However, the disadvantage is that conclusion about causal relationships cannot be made. Cross-sectional research does not provide information about intra-individual change and its inter-individual differences which are the focus of longitudinal studies.

The survey part of the present thesis is based on self-reported data assessed by electronically administered questionnaires. Some general methodological aspects of the survey part of this thesis are discussed below.

Study population

Our study population included dentists and dental hygienists working in PDHS in Norway in 2017. The PDHS is governed and financed by the state. In this regard, policy makers are the ones who regulate which dental materials (and NMs) would be used by dentists. However, personal choice as to what type of dental materials to use is also made by a single dentist. In contrast, dentists employed in the Private Dental Health Care Service in Norway are more independent in terms of their choice of dental materials, but our study has covered only the public sector.

The majority of participants were females, which reflect the common trend in gender distribution among dental professionals in Norway. The age of participants ranged from 22 to 70 years. Nearly half of the dentists were below the age of 36, while the majority of dental hygienists were above 36. Almost half of the participants had 6 to 20 years of working experience.

Structural equation modelling (SEM)

SEM is an advanced statistical technique which is a combination of CFA and Path Analysis. It is widely used in social sciences due to several advantages over conventional analysis, such as multiple logistic regression [199]. SEM allows simultaneous testing of complex relationships between both observed and latent variables in theoretical models, which with the use of other methods would require several separate analyses. Another advantage is its ability to account for measurement error, allowing to make non-biased conclusions about the relationships between latent variables.

Comments on reliability

Reliability refers to the precision or internal consistency of a measurement instrument [200, 201]. A commonly used measure of internal consistency is the Cronbach's alfa coefficient, which indicates how well all the items in the instrument reflect the underlying construct. It is expressed as a number between 0 and 1. For instruments that are used as research tools for comparing groups acceptable values of the coefficient are set to 0.7-0.8 [202]. However, for the clinical application higher values are desirable (minimum 0.90). In the present study, Cronbach's alpha values for the five constructs ranged from 0.80 for perceived behavioral control to 0.93 for attitudes and intention, indicating acceptable and high internal consistency. However, high coefficient value could also be a result of excessive number of items included in the scale measuring the construct. For example, attitudes were measured with 9 items which may result in a high value of Cronbach's alpha due to the large number of items.

Reliability measures in this thesis were limited to internal consistency reliability assessed by Cronbach's alpha, whereas the stability aspect of reliability (test-retest) assessed by comparing the same measure for the same sample across time was not presented. Reliability could be compromised by coding errors and logical inconsistencies of responses. To minimize these errors a uniform 7-point Likert scale was used throughout the questionnaire and pilot-test was performed prior to the study.

Internal Validity

Internal validity refers to how well the instrument (questionnaire) measures what it is intended to measure [200, 201]. Validity of self-reported measures can be examined in terms of three conceptual frameworks - content validity, construct validity and criterion-related validity.

Content validity refers to inclusion of the questions that are representative of the qualities that the instrument intends to measure. In order to ensure content validity, the questionnaire in our study was developed in accordance with the recommendations for TPB questionnaires. Specifically, the principle of compatibility was applied by measuring all the theoretical constructs at the corresponding levels of specificity to obtain strong inter-construct associations. One of the recommendations for TPB questionnaire includes initial qualitative assessment of salient beliefs from the study population, which serve as building blocks for the theoretical predictors of behavioural intention. Although the present study lacks this assessment of salient beliefs, the current TPB questionnaire has been used in a similar study focusing dental students in Norway and Romania [96]. In addition, the questionnaire was pilot tested in a public dental clinic in Bergen to ensure that the included questions were comprehensible. We assume that the abovementioned measures secured a high level of content validity.

Construct validity refers to the extent to which the instrument measures the target constructs [203]. Two fundamental aspects of construct validity include convergent and discriminant validities [204]. Convergent validity is achieved if the items which are supposed to measure an underlying construct are related to that construct. Discriminant validity is achieved when the items which are not supposed to measure a construct are not directly related to that construct [205]. In the present study (Paper II) construct validity was tested with the help of CFA. Values of standardized correlation coefficients below 0.85 and good model fit indicated that proposed model has achieved construct validity.

Criterion validity refers to how well the obtained results correspond to those obtained with the well-established tests (concurrent validity) or to how well the relusts predict

future perforemance (predictive validity) [206, 207]. The results of the present study corresponds with those of a previous study aimed to assess intention to use NMs in dentistry among dental students in Norway and Romania, indicating concurrent validity [96]. Predictive validity of the questionnaire could not be assessed, as actual behavior (use of NMs in dentistry) was not measured in the present study due to the lack of time and resources to inlcude a temporal aspect in the study design. However, according to meta-analyses, intention is considered a good predictor of behavior and has explained on average 19% to 35% of the variance in actual behavior [51, 208, 209].

Validity of self reports in health research can be threatened by information bias, namely social desirabilty and recall bias [210]. In the present study, participants might have considered positive attitudes towards NMs and intention to use NMs in the future as socially desirable answers, which could bias ther responses. To avoid social desirable answers, a short introduction was provided to participants, to inform or remind them that NMs can posess both benefical and non-beneficial characteristics. In addition, the survey inlcuded self-reported question on the amount of knowledge about nanotechnology and a small knowledge test regarding the use of nanothechnolgy in dentistry. The results of subjective and objective knowledge assesement corresponds with each other, implying that social desirability bias regarding knowledge was limited.

Recall bias, which is the aibility to accurately recall past events, can also pose a risk to internal validity. To overcome recall bias, it is generally recommended to select appropriate recall periods. In our survey, participants were asked whether they have used NMs for patient treatment in the past. We assume that the formulation of this question helped to minimise recall bias. However, not all respondents might have been aware that they used NMs, simply because of not knowing that the dental material contained nanoparticles. To minimize those errors, an introduction to the survey included information about the application of NPs in dentistry and illustrations of commonly used dental filling material and dental adhesive with NPs.

External Validity

External validity refers to whether the findings of the study could be generalized to a broader population [211]. Non-response, if occurring not at random, is a major threat to external validity in surveys [212]. Response-enhancement strategies include reminders, incentives, lottery, providing shorter version of the questionnaire, personalization of the invitation to participate in the survey and some others [213]. Studies in the field of nanotechnology report 25% to 76% response rate, pointing that the response rate in the present study (47.5%) is moderate [78, 90, 99, 214]. In addition, dental health care workers in one county (n = 163) did not participate in the survey due to lack of support from the chief dentist. Moreover, although a census of dentists and dental hygienists were invited to participate, responding to the survey was voluntary, which might lead to self-selection attrition bias. It is likely that dental health care workers, who were interested in the topic of nanotechnology or had some knowledge about NMs were more likely than their counterparts to reply to the survey. In this case nonresponse would not be random. In order to minimize self-selection and nonresponse bias a short introductory part was provided to participants which contained definition of nanotechnology, its applications in everyday life and information about both beneficial and non-beneficial features of NPs. The information was presented in a neutral way to avoid any intervention effect. In addition, participants were informed that there were no right or wrong answers to the survey and that all responses were equally valuable. Furthermore, the main invitation to the survey was followed by three reminders and a lottery was drawn among respondents to increase the response rate.

Despite the moderate response rate, gender, age and professional distribution of the respondents was consistent with that of the census of dental health care professionals, as shown in the Results (Tables 5 and 6). This indicates that nonresponses might not have biased the study group considerably and, probably, did not violate the external validity to a large extent. The relatively large number of participants reduces the likelihood that the characteristics of the target population are not represented in the survey group. Taking into account the abovementioned, the study sample could be considered highly representative for the population of Norwegian dentists and dental hygienists employed in the PHDS. It is also important to note that studies that aim to

examine associations (like the present studies I and II) are less strict in terms of requirement of representativeness of the sample compared with studies estimating incidence or prevalence of phenomena.

5.1.2 Study III

TEM

TEM is a powerful tool to study NPs morphology and their interactions with cells. It provides high resolution nanoscale images and, thus is widely used in NPs research [215]. The images are obtained due to transmission of electrons through a thin specimen, which allows the detailed visualization of cell compartments. The disadvantages of this method are that sample preparation is time- and cost-consuming and that the obtained information is static, compared to other *in vivo* imaging techniques which have, however, low resolution [215].

Impedance-based analysis

Testing of NPs' toxicity requires techniques that are not affected by NM-caused interferences and that can provide non-biased results. Traditional methods used in toxicology, such as cell viability assays, *e.g.*, MTT, MTS and WST-8, fluorescent-based flow cytometry and oxidative stress assays, *e.g.*, DCFH-DA, DHE and MBCl, have been shown to be affected by interferences between NPs, components of the assay and/or detection systems [144, 152, 216-218]. In addition, labeling of NPs may change their physicochemical properties leading to biased results that do not reflect real-life effects of NPs. The abovementioned problems could be avoided by using label-free methods, such as impedance-based monitoring, which is non-invasive and can assess the state of cells in real time, compared to other techniques, which only assess toxic effects at a specific timepoint [184, 219, 220]. The assay measures the electrical properties of the cells. More specifically, it measures how well the cells impede the alternating electrical current at low frequencies. These measurements can provide information about a variety of cell properties, such as size, morphology, proliferation, apoptosis and cell-substrate attachment quality [184].

Immunohistochemistry

Immunohistochemistry is a well-established method for the identification and visualization of antigens in tissues based on specific antigen-antibody reaction [221, 222]. This method is used to characterize various biological processes, such as proliferation, differentiation, apoptosis *etc*., and it is widely used in biological research and clinical diagnostics [223]. Our study aimed to examine biological effects, namely proliferation, apoptosis, and tissue integrity, in 3D RNHBM models exposed to two types of TiO_2 NPs at various concentrations. For that purpose, immunohistochemistry served as an appropriate tool. The markers of proliferation, apoptosis, and tissue integrity were Ki-67, cleaved caspase-3, and E-cadherin, respectively. Before conducting the study, all three antibodies were validated by establishing optimal dilution of primary and secondary antibodies using appropriate positive and negative controls. A positive control is a tissue known to express the antigen of interest. In our study, tonsil tissue served as a positive control for Ki-67 and E-cadherin antibodies, and a 3D RNHBM model, exposed to sodium lauryl sulphate at high concentration was used as a positive control for cleaved caspase-3 [151]. For negative control 3D RNHBM samples were used in which primary antibody was substituted with diluent.

Image analysis

The quantification of the staining was performed with the help of QuPath - an open source bioimage analysis platform designed for whole slide images. This software was shown to be a reliable tool that provides accurate and reproducible results [191, 224-227]. In the present study, the process of quantification was semi-automated. First, the RNHBM models were manually annotated by one researcher after several sessions of calibration with an oral pathologist. Then the optimization of cell detection was performed by setting a threshold for tissue background, Hematoxylin and Eosin staining and DAB staining for each antibody. This allows QuPath to classify each pixel of the image as positively/negatively stained based on a threshold value. The threshold was further applied to all images within the relevant antibody, allowing automated cell detection. The advantage of this method is that all images within one antibody are processed equally based on pixel values and, thus it is free from researcher subjectivity.

It also allows to perform the analysis much quicker compared to manual quantification. The limitation of the technique is that it requires manual assessment of the validity of automated quantification when it is applied to all tissues, as differences in tissue processing, staining, and imaging could result in under- or overdetection of cells.

Cell culture

Since inhalation and ingestion are important routes of TiO_2 NPs exposure, lung cancer epithelial cells (A549) and primary normal oral fibroblast were used in the study. A549 was purchased for this study as it is the most commonly used cell line in inhalation toxicology [228]. Normal oral fibroblasts were primary cells obtained from the patients. The advantage of using primary human cells is the higher relevance to *in vivo* conditions compared to immortalized cell lines. It was reported that primary cells preserve better the characteristic behavior of cells including differentiation process and the cross-talking, while cell lines usually harbor genetic alterations and often exhibit atypical cellular behavior [229]. However, isolating cells requires special skills and is more time- and cost-consuming compared to purchased cell lines. Cells in monolayers, both primary and cell lines, could serve as a reliable tool for initial toxicity screening. However, results of *in vitro* studies on 2D cell cultures have poor generalizability to human body as they lack the biological microenvironment, *e.g.*, neighboring cells, vascularization, immune component, which can alter cell responses to NP exposure [228]. In this regard, in-depth mechanistic studies, performed on more advanced biological models, can provide results that reflect more closely real-life exposure.

Reconstructed Normal Human Buccal Mucosa Model

In order to improve the generalizability of *in vitro* testing for the *in vivo* context, 3D models have been developed and have shown promising results. The advantage of the 3D RNHBM model is that it mimics the multilayer structure of buccal epithelium and underlying connective tissue. In addition, because it contains two cell types (keratinocytes and fibroblasts) it allows epithelial-mesenchymal interaction, which plays an important role in the development and regeneration of oral mucosa [159, 230, 231]. Another advantage is that a large number of RNHBM models (20-30 on average) can be generated from one donor tissue, allowing testing of multiple conditions. The

limitations of 3D models are limited lifespan and big range of morphological variations, such as epithelial thickness and differentiation, between the models generated from different patients. They are also more time-consuming and labour-intensive compared to 2D cell cultures. RNHBM models that were produced from one donor tend to be more similar. In this regard, to account for possible correlations between the samples arising from one donor, intraclass correlation coefficient was estimated in the present study. It is also important to note that in the oral cavity, the epithelium is moistened with saliva. Its presence and composition, for example PH level, can influence agglomeration state of NPs and change their toxicity [154]. In the present study, saliva was not introduced into the model, nor blood or lymphatic vessels or components of the immune system.

5.2 Discussion of main results

5.2.1 Study I and II

Risk and benefit perceptions

The present study revealed that more than half of Norwegian dental health care workers has both high benefits and high risks perceptions associated with the use of dental NMs. This accords with the previous studies in the US, Europe and Asian countries [72, 75, 232]. Overall, previous experience with NMs, feeling safe to use them, having trust in stakeholders and less work experience was associated with increased likelihood of benefit perceptions. Having correct knowledge about NMs, being worried about the increasing use of these materials and having high trust in stakeholders was associated with increased likelihood of risk perceptions.

Among sociodemographic factors, employment status was significantly associated with risk and benefit perceptions. Specifically, dentists were more likely than dental hygienists to perceive both high risks and high benefits associated with the use of dental NMs. This might be explained by the difference in education of the two employment groups, with dentists having 5-years master and dental hygienists a 3-years bachelor degree. This is supported by results from previous studies that have shown that people with higher level of education tend to give greater support for nanotechnology

compared to respondents with lower education [72, 233]. Another possible explanation of higher risk perceptions among dentists than dental hygienists is the fact that dental hygienists are using a much narrower range of dental materials compared to dentists. Thus, dentists are considered to have higher familiarity with dental NMs which might lead to more critical assessment of both risk and benefits associated with the use of such materials.

Dental health care workers with less work experience were most likely to report benefit perceptions. A possible explanation could be that participants with less work experience were those who have recently graduated from dental schools and might have become more familiar with dental NMs during their education as the topic of NMs have become a part of dental curriculum. In addition, past experience with dental NMs was also positively associated with higher benefits perceptions. This suggests that dental health care workers have derived benefits from previous use of dental NMs and, thus were more supportive compared to those who have not used or were not aware that they have used NMs previously. This finding is supported by a recent study on consumer acceptance of nano clothing, which found that previous experience with nano clothes was associated with perceived usefulness of such clothes [234].
The present study did not reveal any strong effect of gender on dental health care workers' risk-benefit perceptions. This contradicts with the results from previous studies, reporting women to have higher risk perceptions compared to men [73, 75, 235, 236].

Knowledge about the application of nanotechnology in dental practice was assessed by means of a single item (self-reported knowledge) and a multi-item knowledge test (correct knowledge). Half of the respondents reported that they have moderate/much/very much self-reported knowledge, and slightly more than half demonstrated moderate/much correct knowledge about the use of NMs in dentistry. This finding is consistent with previous studies revealing that half of the participants, who were general public from USA, Europe and Japan had no familiarity with nanotechnology [72]. It has previously been suggested that knowledge might be a factor influencing risk-benefit perceptions. The present study revealed that dental

health care workers with more correct knowledge were more likely to have high risk perceptions. Probably, greater familiarity with dental NMs implies greater awareness about potential risks associated with their use. Some authors claim that participants with greater familiarity about nanotechnology perceive more benefits and less risks, while others suggest that knowledge does not have any effect on risk-benefit perceptions [72, 76, 79].

The results of the present study showed that trust in stakeholders was associated with both high benefit and high risk perceptions. This finding is in line with the results of a previous study, which has reported that trust in government increased both benefits and risk perceptions of nanotechnology among general public in Malaysia [232]. In contrast to our findings, previous studies have shown that higher trust in different stakeholders like scientists, journalists, politicians, industry and business leaders is associated with higher benefit and lower risk perceptions [72, 73, 75, 76, 78, 235].

The present study revealed that most of the participants exhibited high benefits and high risk perceptions. A recent study assessed how the type of information processing (affect- or cognition-based) might influence the relation between risks and benefits perceptions across different technologies [237]. The authors concluded that, participants with cognition-based information processing judged benefits as high and risks as low (negatively related), while participants with affect-based information processing perceived both benefits and risks above average (positively related). It is also known that public responses to emerging technologies are mostly driven by affect rather than cognition [99]. This might be an explanation for high benefit and high risks perceptions among participants in the present study. The hypothesis of affect-driven response in our study was also supported by the fact that benefit perception was strongly associated with feeling safe to use NMs, while high risk perception was associated with high level of worry about the increased use of dental NMs.

Intention to use NMs

Our study revealed that attitudes were the strongest predictor of the intention to use NMs followed by perceived behavioral control and subjective norms. Thus, dental

health care workers with more positive attitudes towards the use of NMs, with more confidence about managing such materials and greater perceived social pressure had stronger intention to use these novel materials. Previous research has shown that the relative importance of the three TPB constructs varies across groups of clinicians as well as across different behaviors [238]. Similar to the present study, attitudes and perceived behavioral control have been identified as the strongest predictors of various behavioral intentions among dental health care workers' [58-60, 62, 66, 239]. Subjective norms were the weakest, but still a significant predictor of intention in our study, indicating that perceived social pressure was less influential compared to attitudes and perceived behavioral control. The relative importance of subjective norms varies greatly across different behaviors of dental health care workers from being the strongest predictor of intention to manage drug users [65] to not having any influence on intention to place fissure sealants [59] and intention to advise on diet, tobacco and alcohol consumption [63].

Two external variables in terms of past behavior and risk perceptions were incorporated into the TPB model in our study. Previous research shows contradictory results with regards to past behavior [240]. In our study, past behavior had both direct and indirect (through the TPB constructs) positive effects on intention. Participants who had previous experience with dental NMs would rather intend to use them in the future. In addition, they possessed more positive attitudes, stronger perceived behavioral control and greater perceived societal pressure and, thus had stronger intention to use these materials in the future.

Several researchers have augmented the TPB model with perceived risk using it as an external variable. Across the studies, perceived risk had mostly moderate to strong relation to health behaviour, however, negative or no relation was also reported [241]. In our study there was no direct relation between risk perception and intention, suggesting that this relation is more complex than it was hypothesized originally. However, risk perception had an indirect effect on intention through attitudes and perceived behavioral control, indicating that participants with low risk perceptions had

more positive attitudes and stronger perception of control over their decision to use NMs, which, in turn, were associated with higher intention to use such these materials.

The results of **Study I** could be used for evidence-based communication of risks and benefits associated with the use of NMs in dentistry. Specifically, it was demonstrated that dental professionals' perceptions of risk and benefits are affect-driven. Thus, when communicating risks to this professional group attention should be paid to such factors as feeling safe and being worried about the use of NMs in dentistry. The fact that survey respondents were aware of both benefits and risks associated with the use of dental NMs, implies that dental professionals in PDHS in Norway possess balanced information and thus are not expected to have strong opinions which can promote opposition to the use of dental NMs. However, with the appearance of new toxicological evidence and new regulations, such as a recent ban on the use of TiO_2 NPs in food, risk-benefit perceptions could change in a negative direction [11, 140]. In this regard, constant monitoring of public, experts' and stakeholders' risk-benefits perceptions is of high importance.

The results of the **Study I** have also demonstrated the need for more information about applications of nanotechnology in dentistry. A moderate level of knowledge about NMs among dental health care workers indicates that there might be a need for dental curriculum modification, or for introduction of postgraduate educational courses on the use of NMs in dentistry.

The results of **Study II** have revealed the important predictors of dental health care workers' intention to use NMs for patient treatment in the future. Specifically, attitudes, perceived behavioral control, subjective norms, past behavior and to some extent risk perceptions should be considered by policy makers when communicating risks to dental health care workers and managing risks related the use of dental NMs.

5.2.2 Study III

Agglomeration effects of the TiO_2 NPs

When in contact with water or other solutions and biological liquids, TiO_2 NPs tend to form agglomerates of various sizes. This might influence their uptake into cells and

their toxicity. The results of the studies are often contradictory and depend on several factors, such as concentration of NPs, primary particles size, presence of FBS and cell types used [130, 144, 242-244]. Moreover, only a few studies satisfy the minimum physicochemical characterization requirements, which makes it difficult to assess which physicochemical characteristics are responsible for the observed effects. In the present study, spherical NPs had a greater tendency for agglomeration compared to the spindle-shaped ones. However, there was no difference in the biological effects of larger agglomerates of spherical NPs compared to smaller agglomerates of spindle-shaped NPs in 2D cultures, as detected by impedance-based monitoring and in 3D RNHBM models, as detected by immunohistochemistry. Our findings contradict the results of a recent study that reported increased cytotoxic effect of less agglomerated TiO_2 NPs (spherical, 30 nm, anatase) on A549 cells compared to highly agglomerated ones (spherical, 21 nm, 90% anatase and 10% rutile) [129]. The difference in the findings could be due to the different crystalline form of NPs used, other physicochemical characteristics or methodological differences.

Shape effects of the TiO_2 NPs

More information is needed regarding the shape effects of TiO_2 NPs on their putative toxicity. In the present study, no difference in the response of cell culture models exposed to spherical and spindle-shaped TiO_2 NPs was detected. In contrast to our study, results of Kose et al. (2020) suggested that rod shaped TiO_2 NPs (20 - 250 nm, anatase) caused more cell death in A549 cells compared to spherical NPs (21nm, 90% anatase and 10% rutile) after exposure to a concentration range of 15 - 120 µg/ml [129]. Similar effects were reported by Gea et al. (2019), who exposed bronchial epithelial cells to differently shaped TiO_2 NPs at a concentration range of 5 - 80 µg/ml and found that rod shaped NPs (108±47, anatase) exhibited higher cytotoxicity compared spherical TiO_2 NPs (20 ±5 nm, 90% anatase and 10% rutile) [245]. The results of abovementioned studies could not be directly compared to the results of the present study, due to differences in NPs' size (much larger length of the rods) as well as crystalline structure (anatase is generally considered more toxic than rutile).

Effect on 2D cell cultures

The effects of TiO_2 NPs on different cells have been extensively studied, sometimes resulting in contradictory results. Some authors report clear cytotoxic effects, while others little or no effect at all [126, 243, 246-248]. Rutile TiO_2 NPs at concentrations range of 0.25 - 1.0 mg/ml were shown to significantly reduce the viability of gingival fibroblasts [247]. However, in another study, anatase TiO_2 NPs (primary particle size 25 nm) at a concentration range of 0.05 - 3.2 mM did not affect cell viability of oral fibroblasts but led to an increase in production of prostaglandin E_2 [246]. It was also revealed that TiO_2 NPs with a primary particle size of 30 nm could cause medium cytotoxic effects in A549 cells at concentrations between 50 - 200 µg/ml [248]. However, Cho et al. (2013) did not find any change in the viability of A549 cells exposed to rutile-TiO_2 NPs (primary particle size 30.5 nm) at 30 – 300 cm^2/mL [243]. Similarly, no effect of TiO_2 NPs (anatase/rutile, 30 nm primary particle size) at 1-100 µg/ml on cells viability of A549 was observed after 24 h of exposure [126]. Presumably, differences in protocols, such as physicochemical properties of NPs, dispersion of NPs, exposure conditions, composition of cell culture medium *etc.,* could explain the dissimilarity between study results. Moreover, conventional toxicity screening methods, such as MTT, are known to be more prone to interferences from NPs, which might lead to biased results. Furthermore, the information provided in the studies with regard to the physicochemical characteristics is oftentimes minimal.

In our study, exposure to both spherical and spindle-shaped TiO_2 NPs led to dose-dependent decrease in cell proliferation of oral fibroblasts at 80 and 100 µg/cm^2. However, no changes were observed in the epithelial lung cancer cells. The difference in the cell response to TiO_2 NPs might be due to the fact that oral fibroblasts were normal primary cells, compared to A549 cells, which is an immortalized cancer cell line with higher proliferation rate. The latter are also more resistant to external stimuli [249].

Effects on 3D RNHBM

Oral cavity is subjected to exposure to TiO_2 NPs from food, medicines, and oral care products. However, very little research is available regarding possible adverse effects

of TiO_2 NPs on oral tissues. We have previously demonstrated that TiO_2 NPs could penetrate the upper layers of epithelium of 3D RNHBM models [165]. Another study has revealed that TiO_2 NPs can cross porcine buccal mucosa and translocate to submandibular lymph nodes [169]. In addition, several studies on 2D co-cultures of intestinal epithelial cells have confirmed cytotoxic effects of TiO_2 NPs, while others reported little or no toxicity [6, 141, 142].

Our present study evaluated biological effects induced by TiO_2 NPs on the epithelium of 3D RNHBM. Although it was demonstrated that the highest concentrations of both spherical and spindle-shaped TiO_2 NPs led to a slight decrease in proliferation and an increase in apoptosis in epithelium, these changes were not statistically different from control. A possible explanation could be the biological variation between the tissues derived from different patients and the small sample size. Moreover, epithelial cells might have different responses to external stimuli – either differentiation or programmed cell death [250]. Thus, exposure to TiO_2 NPs could have accelerated the differentiation of keratinocytes in the epithelium of RNHBM without influencing the proliferation and/or apoptosis processes. Accelerated differentiation could lead to increased desquamation, which was observed in the present study in exposed 3D RNHBM models, but it was not possible to quantify it. To confirm the differentiation of epithelial cells, biological markers of differentiation should be used in prospective studies.

As mentioned above, a previous study of our group has revealed that the same TiO_2 NPs could penetrate the upper layers of epithelium of 3D RNHBM [165]. In addition, it was shown that exposure to NPs might influence epithelial homeostasis. Specifically, we reported that higher concentrations of TiO_2 NPs induced the desquamation of epithelium, while the thickness of epithelium remained unchanged. It was thus theorized that the desquamation of upper layers was compensated by increased proliferation in the basal layer. Although our current study has revealed an increased proliferation in the basal layer of epithelium in 3D RNHBM models exposed to the highest concentration of TiO_2 NPs, it was not proven to be statistically significant.

In study III, matching concentrations of TiO_2 dispersions (0.2, 1 and 100 μg/cm^2) were used to compare the effect of TiO_2 NPs on oral fibroblasts and on RNHBM, which represent 2D and 3D models, respectively. No effect was observed in 2D and 3D models exposed to the lowest concentration (0.2 μg/cm^2) of spherical and spindle-shaped TiO_2 NPs. The middle and high concentrations (1 and 100 μg/cm^2) led to a dose-dependent decrease in the proliferation in 2D cell cultures but did not induce any changes in RNHBM. The difference in results between 2D and 3D cultures might be explained by better protective mechanisms of 3D models, such as a multilevel structure of epithelium and ability to renew itself (epithelial turnover). On the contrary, cells in 2D culture do not have such protective mechanisms and might be more susceptible to NPs exposure. Nevertheless, NPs testing in 2D cultures is an important step in toxicity testing that could be used as initial screening of the particles. Further tests should be performed in more complex models, such as co-cultures or 3D models, which better resemble human tissues.

6. Conclusion

The overall aim of the present study was to address the issue of nanosafety in dentistry from two different perspectives: (1) assessment of risk/benefit perceptions and intention to use NMs by dental professionals and (2) evaluation of *in vitro* cytotoxic effects of TiO_2 NPs related to oral and lung exposure using NAMs.

Based on the present findings, the following conclusions can be made:

I. More than half of the Norwegian dental health care workers had both high benefits and high risks perceptions associated with the use of dental NMs. Feeling safe to use NMs was strongly associated with benefit perceptions, while being worried about increasing use of NMs - with risk perceptions.

II. Dental health care workers exhibited moderate to strong intention to use dental NMs. The strongest predictors of intention to use NMs were attitudes and perceived behavioral control, followed by past behavior and subjective norms. High risk perception of NMs was associated with low intention to use these materials.

III. Exposure to both types of TiO_2 NPs impaired the proliferation of normal oral primary fibroblasts, but not of lung cancer epithelial cells. The proliferation, apoptosis, and tissue integrity of 3D RNHBM epithelium were not affected by TiO_2 NPs.

6.1 Future perspectives

The present study employed a well-recognized theoretical framework (TPB) augmented with external variables reported to be influential for the attitudes towards NMs. However, the field of nanotechnology is constantly developing and there might be other factors influencing the intention to use these novel materials. In this regard, future studies should uncover other key-factors predicting intention to use dental NMs.

Study II assessed the intention to use NMs as a proximal predictor of behavior. As suggested by the TPB, the higher the intention the higher the likelihood that the

behavior will take place, however, gaps between those constructs have been identified. Thus, future research should assess subsequent behavior in order to better understand the relationship between actual behavior and its predictors.

The results of Study III imply that epithelium of 3D RNHBM models exposed to TiO_2 NPs might undergo differentiation. This finding should be further examined with the help of differentiation markers.

With regards to toxicity assessment of NPs, impedance-based monitoring has been proven to be an efficient and less prone to NM-interferences tool for initial toxicity screening that can provide useful information regarding relevant time-points and concentration. Further, in-depth studies elucidating the mechanisms of decreased cell proliferation in oral fibroblasts by NPs are required to better understand the significance of the *in vitro* findings. A first step could be the investigation of ROS production in the exposed cells. This might be an important link related to potential risks of premature senescence, aging or cancer development with exposure to NPs.

The 3D RNHBM model is a promising tool with the potential to replace or minimize animal studies. Future model development is needed to overcome the existing limitations, such as lack of vascular and immune system components. Moreover, to better mimic the *in vivo* conditions of oral cavity, a saliva substitute should also be introduced into the model.

The current study employed NAMs in terms of electrical impedance-based monitoring and 3D models for assessment of nanotoxicity. Future research should focus on elaborating standardized protocols to increase the applicability of both methods for the safety assessment of NMs.

6.2 Implications

The results of **Study I** have uncovered several factors associated with risk and benefit perceptions related to the use of dental NMs among dental health care workers. The results of **Study II** have revealed the important predictors of dental health care workers' intention to use NMs for patient treatment in the future. The findings of both studies should be considered by policy makers when communicating risks to dental health care workers and managing risks related to the use of dental NMs. In addition, a moderate level of knowledge about NMs among dental health care workers might indicate a need for revision of current dental curriculum or for introduction of postgraduate educational courses on the use of NMs in dentistry.

The results of **Study III** add to the knowledge regarding possible effects of TiO_2 NPs on cell viability and proliferation *in vitro* reflecting oral and lung exposure. The fact that oral fibroblasts exhibited decreased proliferation after 24 h of exposure with spherical and spindle-shaped TiO_2 NPs raises concerns. Although under real life exposure the NPs would first come in contact with oral keratinocytes in healthy epithelium, they could still reach oral fibroblasts either by penetrating through epithelium or by direct contact in case of oral mucosa injury. Thus, this finding requires future investigation to elucidate the mechanisms behind the decrease in proliferation of oral fibroblasts after exposure to spherical and spindle-shaped TiO_2 NPs. In addition, the present results of both 2D and 3D *in vitro* testing could be used in *in silico* approaches aiming to predict potential toxicity of newly developed materials.

7. Source of data

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8. Original papers

Paper I

I

Use of nanomaterials in dentistry: Covariates of risk and benefit perceptions among dental health care workers in Norway

Xenaki, V., Costea, D. E., Marthinussen, M. C., Cimpan, M. R., and Astrom, A. N.

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Paper II

Predicting intention of Norwegian dental health-care workers to use nanomaterials: An application of the augmented theory of planned behavior

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II

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ORIGINAL ARTICLE

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Predicting intention of Norwegian dental health-care workers to use nanomaterials: An application of the augmented theory of planned behavior

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Abstract

Due to the rapid development of nanotechnology and its integration into dentistry, there is a need for information on the factors influencing the decision of dental health-care workers to use nanomaterials. Based on a national survey among Norwegian dentists and dental hygienists, this study applied the theory of planned behavior (TPB), augmented with past behavior and perceived risk, to predict the intention to use dental nanomaterials in the future and to assess whether an augmented TPB model operates equivalently across professional groups. Structural equation modelling was used to assess whether the hypothesized model fits the data. Of 1792 eligible participants, 851 responded to an electronic survey. Attitudes and perceived behavioral control had the strongest effect on intention, followed by past behavior and subjective norms. Risk perceptions had an indirect effect on intention. Multigroup comparison confirmed invariance of the model across professional groups. This study supports the validity of the augmented TPB model to explain the intention of Norwegian dentists and dental hygienists to use nanomaterials. The strongest influence on intention is given by the attitudes toward nanomaterials and perceived confidence in their use. The findings of the study have implications for management of the use of nanomaterials in dentistry by policy makers.

KEYWORDS
attitudes, behavioral research, dental nanomaterials, intentions, structural equation modelling

INTRODUCTION

Nanotechnology is one of the essential technologies of the 21st century [1]. It involves the use of nanomaterials, which are defined as 'natural, incidental, or manufactured materials containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm' [2]. As a result of the unique properties of nanoparticles, nanotechnology has become a

promising field that has improved many aspects of human life. However, nanoparticles may also exhibit toxic effects and this raises concerns about possible health and environmental risks [3]. A significant body of research has focused on the unique properties of nanoparticles, their toxicological aspects [4, 5] and the development of reliable tools for assessment of nanotoxicity [6, 7]. By contrast, relatively little research has been carried out regarding the opinions of stakeholders and the general public on nanotechnology and the intention to use innovative materials.

Studies from Europe and the United States have demonstrated that the general public is rather unfamiliar with the topic of nanomaterials [8–11] and that their attitudes toward nanotechnology are either positive or neutral [8, 11, 12]. Moreover, there is evidence indicating that risk perceptions related to nanotechnology are higher among laypersons than among nanotechnology experts, policy makers, and risk managers [9, 10, 13–15]. However, possible environmental pollution and long-term health problems associated with nanotechnology, as well as use of nanomaterials in food, cosmetics, and packaging, have raised higher concerns among scientists than among non-experts [14, 15]. Interestingly, a recent study revealed that nano-scientists and engineers perceive lower risk than the experts involved in risk regulation and management [16]. Considering that nanotechnology is a relatively new and continuously developing field, the opinions of stakeholders and the general public have not been completely established and thus might change in pace with accumulation of new knowledge [14].

Dentistry is among the fields that have been significantly improved by nanotechnology [17]. The current market offers a variety of dental materials modified by nanoparticles, such as restorative composites, glass ionomer cements, adhesives, and bone-regenerative materials, to name but a few [18–20]. Recently, it has been demonstrated that dentists and dental hygienists have moderate knowledge about nanomaterials and perceive both risks and benefits related to their application [21]. Although several studies have reported on public and expert opinion about nanotechnology, few studies have investigated the attitudes of dental health-care professionals toward this technology [8–16]. Thus, our understanding of the reasons why dental health-care workers use or refrain from use of nanomaterials in the context of clinical dental care is incomplete. Investigation of the attitudes of dental health-care workers towards nanomaterials is essential because it plays an important role in their acceptance or rejection of nanotechnology [22, 23]. To assist policy makers in their management practice, we need to identify the psychosocial factors that influence the decision of dental health-care workers on whether or not to use nanomaterials when treating patients in the future.

The theory of planned behavior (TPB) is a well-recognized theoretical framework of the attitude–behavior relationship, which assumes that most conscious behaviors are goal directed [24]. This theory is an extension of the theory of reasoned action (TRA) and has been applied across various populations, contexts, and behavioral domains [25–31]. In addition to the TRA constructs, the TPB includes perceived behavioral control, therefore allowing a better explanation of behaviors which are beyond full volitional control and improved predictive power of the model [24, 32]. Moreover,TPB has proved to be a reliable tool in predicting and explaining occupational behaviors [26, 30, 31, 33–36]. A systematic review revealed consistency of predicted behavior between health-care professionals and non–health-care professionals, indicating that TPB is a valid tool for use in the occupational context of health care [26]. Meta-analyses have shown that the TPB explains (on average) 39%–59% of the variance in behavioral intention, whereas intention explains (on average) 19%–35% of the variance in actual behavior [30, 37, 38].

According to the TPB, behavior is predicted by behavioral intention (summarizing a person's motivation to engage in a particular behavior and indicating how hard the person is willing to try and how much time and effort he or she is willing to devote in order to perform the behavior) and perceived behavioral control (perception of presence or absence of necessary resources and opportunities as well as anticipated obstacles or impediments related to performing the behavior). Intention, in turn, is a joint function of perceived behavioral control, attitudes toward performing the behavior (positive or negative evaluation of the behavior), and subjective norms (perceived social pressure of performing or not performing the behavior). The TPB maintains that the relative importance of the TPB constructs differs according to the particular behavior and populations investigated [32].

As proposed by Ajzen [32], the original TPB model can be augmented by external variables, such as demographics, moral norms, descriptive norms, and anticipated regret, in accordance with the context and nature of the particular behavior investigated [25, 30, 39]. A number of studies have reported on residual effects of past behavior on intention and future behavior after having controlled for the original TPB constructs, suggesting that these effects reflect the sufficiency of the TPB model [40, 41]. Only a few studies have considered the occupational behavior of dental health-care professionals using a socio-cognitive approach [33, 34, 36, 42, 43].

Whereas knowledge was demonstrated to be an important covariate of the risk perceptions of dental health-care workers related to use of nanomaterials[21], a socio-cognitive model to explain variance in intention to use these materials has yet to be validated among dentists and dental hygienists employed in the public dental health-care service in Norway. As dental health-care workers have been using dental nanomaterials for patient treatment, it seems relevant to investigate whether past behavior predicts the intention to use nanomaterials beyond the effect of the original TPB constructs. In addition, risk perceptions related to nanomaterials might influence behavioral intention, as demonstrated by previous studies

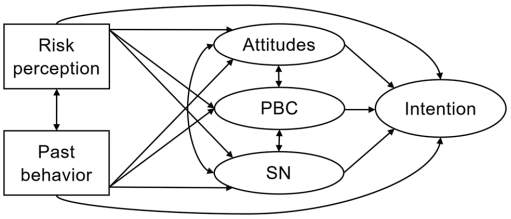

FIGURE 1 The hypothesized augmented theory of planned behavior (TPB) model including four latent variables (intention, attitudes, perceived behavioral control [PBC], and subjective norms [SN]) and two observed variables (risk perception and past behavior)

[27–29]. Relying on the TPB augmented with past behavior and risk perception, the purpose of this study was to predict the intention of dental health-care workers to use nanomaterials in the future and to explore whether the augmented TPB model operates equivalently across the professional groups of dentists and dental hygienists. In accordance with TPB, it was hypothesized that positive attitudes toward the use of dental nanomaterials, stronger confidence in the ability to use these materials (perceived behavioral control), and higher pressure from significant others (subjective norms) increase the intention to use dental nanomaterials. Furthermore, it was suggested that external variables, in terms of risk perception and previous experience with nanomaterials (past behavior), have both direct and indirect effects on behavioral intention, through attitudes, subjective norms, and perceived behavioral control. The hypothesized model for the present study is depicted in Figure 1.

MATERIAL AND METHODS

A census of all dentists and dental hygienists working in the public dental health-care service in Norway (1792 eligible participants) was asked to participate in a cross-sectional self-administered survey in March–May 2017. The questionnaire was developed based on recommendations for TPB questionnaires and relevant literature [44] and was pilot-tested in a public dental clinic in Bergen. The Norwegian Centre for Research Data approved the survey (51053/3/AMS) and was responsible for administration of the questionnaire, data collection, and anonymization of personal information about participants. The questionnaire, together with the informed consent and a short introductory description of nanomaterials (Appendix S1), was distributed by e-post. The main invitation to the survey was supplemented by three consequent reminders in an attempt to increase the response rate.

The questionnaire included the original constructs of the TPB: intention, attitudes, perceived behavioral control, and subjective norms. Each of the TPB constructs was measured by several items, with responses recorded on a seven-point Likert scale that ranged from '1 = strongly agree' to '7 = strongly disagree' (except for item 18 that ranged from '1 = very easy' to '5 = very difficult'). The scales of items 7, 10, and 12 were reversed as they represented negative statements (Table 1). Low scores indicated positive cognitions, and high scores indicated negative cognitions. Intention was measured by four items, three of which assessed positive intention, while the fourth asked about the likelihood of using nanomaterials in the future. Attitudes were measured by nine items: six were positively worded and three were negatively worded. Perceived behavioral control and subjective norms were measured by five and four positively phrased items, respectively. In addition, two variables, external to the TPB model, were incorporated: (1) risk perceptions of dental nanomaterials, and (2) past behavior. Risk perception was a summative score of six items, each assessed on a seven-point Likert scale that ranged from '1 = very likely' to '7 = very unlikely', for which low scores represent high perception of risk and high scores represent low perception of risk (Table 1). Past behavior was measured by one item 'Have you used dental nanomaterials for patient treatment before?' with response alternatives '1 = yes', '2 = no', and '3 = I don't know', which were further dichotomized into '0 = yes' and '1 = no/I don't know' for the purpose of analysis. In accordance with recommendations, the TPB constructs in the augmented model were measured considering the four elements of action (using), target (nanomaterials), context (for patient treatment), and time (in the future) [24].

Statistical analysis

Descriptive statistical analysis, in terms of frequencies and mean distributions, was conducted using SPSS, version 25.0 (IBM). Structural equation modelling was performed using the Lavaan package [45] in R (R Core Team). Structural equation modelling is an advanced statistical technique that enables us to investigate whether the hypothesized augmented TPB model has acceptable fit to the data, testing simultaneously the interrelationships between the constructs specified in the hypothesized model [46].

In the present study, a two-stage modelling approach was used to test the hypothesized augmented TPB model [47]. First, confirmatory factor analysis (CFA) was performed to test the factorial validity of the latent constructs and the adequacy of the measurement model. In the first stage, four latent constructs comprising the original TPB model were used (intention, attitudes, perceived behavioral control, and subjective norms), excluding risk perception and past behavior as they were used as observed variables in the model. Potential sources of misfit were examined with the help of modification indices, which provided a basis for the re-specification of the measurement model.

TABLE 1 Descriptive statistics for the theory of planned behavior (TPB) measurement model

Latent factor	Itemno.	N[a]	Question	Scale	Mean	SD
Intention; $\alpha = 0.93$						
	1	712	I intend to use dental nanomaterials for patient treatment in the future	b	3.2	1.3
	2	715	I plan to use -//-	b	3.2	1.4
	3	712	I have decided to use -//-	b	3.5	1.3
	4	718	How likely is that you will use -//-	c	2.8	1.3
Attitudes; $\alpha = 0.93$						
	5	754	To use nanomaterials for dental treatment in the future is a good idea	b	3.4	1.2
	6	751	-//- is important	b	3.4	1.2
	7[e]	749	-//- is dangerous	b	3.9	1.0
	8	734	-//- is responsible	b	3.5	1.1
	9	729	-//- is reasonable considering the quality of treatment	b	3.2	1.1
	10[e]	735	-//- is irresponsible considering the patient's health	b	3.8	1.1
	11	705	-//- is valuable	b	3.3	1.1
	12[e]	709	-//- is useless	b	3.2	1.1
	13	713	-//- is interesting	b	2.9	1.3
Perceived behavioral control; $\alpha = 0.80$						
	14	668	If I want, I have the possibility to use dental nanomaterials for patient treatment in the future	b	3.0	1.3
	15	673	It is totally up to me if I use -//-	b	3.9	1.5
	16	673	I have all the resources I need to use -//-	b	3.7	1.4
	17	669	I am sure that I am able to use -//-	b	3.2	1.3
	18	672	How easy or difficult you think it is to use -//-	d	2.7	0.7
Subjective norms; $\alpha = 0.87$						
	19	661	Colleagues who influence my clinical practice think that I should use dental nanomaterials for patient treatment in the future	b	3.9	1.2
	20	661	Colleagues who are important to me think that I should use -//-	b	3.8	1.2
	21	655	The chief dentist of my clinic thinks that I should use -//-	b	3.9	1.1
	22	659	The chief dentist of the county thinks that I should use -//-	b	3.8	1.0
Risk perception[f]; $\alpha = 0.89$						
	23	660	How likely is that you subject yourself to health damage by using dental nanomaterials in the future	c	3.9	1.1
	24	657	How likely is that you increase your own risk to get cancer by using -//-	c	4.0	1.1
	25	658	How likely is that you inhale nanoparticles that accumulate in your body if you use -//-	c	3.7	1.2
	26	647	How likely is that you contribute to the uncontrolled spreading of nanoparticles if you use -//-	c	3.6	1.2
	27	649	How likely is that you contribute to patient's health damage if you use -//-	c	4.1	1.1
	28	646	How likely is that you contribute to environmental pollution if you use -//-	c	3.4	1.3

[a]Number of participants does not add up to 851 in the questions because of missing values (11%–24% in separate items).
[b]7-point Likert scale ranging from 1 (strongly agree) to 7 (strongly disagree).
[c]7-point Likert scale ranging from 1 (very likely) to 7 (very unlikely).
[d]5-point Likert scale ranging from 1 (very easy) to 5 (very difficult).
[e]Scale of items 7, 10, and 12 was reversed as they represent negative statements.
[f]Risk perception is a summative score (range 6–42), incorporated as an observed variable in the structural equation model.

Second, following the specification of the measurement model, structural equation modelling was performed to examine whether the hypothesized augmented TPB model has acceptable fit to the data and to estimate direct, indirect, and total effects of relationships in the model. The following statistical parameters were used to measure how well the hypothesized model fit the data – chi-square (χ^2) test, comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR) [48]. A statistically non-significant chi-square test result (i.e., $P > 0.05$) indicates good fit of the model. However, because this test is highly sample-size sensitive (large samples can lead to a significant *P*-value of the chi-square test, even with trivial misspecifications), the emphasis was set on the remaining fit indices. In line with conventional recommendations of Hu and Bentler [49], values of CFI > 0.90 and > 0.95, of RMSEA < 0.08 and < 0.06, and of SRMR < 0.08 and < 0.05 indicate acceptable fit and good fit, respectively. The maximum likelihood estimator with robust standard errors was applied to account for non-normally distributed data. Missing data were handled by the full information maximum likelihood, which is most often superior to handling missing data by use of standard ad hoc routines, such as mean replacement and listwise or pairwise deletion [50].

Multigroup analyses were performed with CFA and structural equation modelling to test whether the model was invariant across the two groups of employees. Before investigating the invariance of predictive paths (using structural equation modelling), the configural and metric invariance was assessed in the final measurement model (using CFA). The configural invariance (equal forms) was tested by fitting the final measurement model across dentist and dental hygienists. Configural invariance was supported if the model had a satisfactory fit (based on the above-mentioned fit indices). Metric invariance (equal factor loadings) was tested by constraining factor loadings in both groups and by comparing the constrained model with the baseline model (configural invariance model) in which factor loadings were free to vary. Metric invariance was supported if the chi-square change was non-significant and the CFI change was less than 0.002 [51]. Invariance of predictive paths was tested by comparing a structural equation model in which both factor loadings and regression paths were constrained across the groups with a baseline structural equation model in which factor loadings were constrained and regression paths were free to vary. The criteria for invariance of predictive paths were insignificant chi-square change and CFI change less than 0.002.

RESULTS

A total of 851 participants responded to our survey (response rate 47.5%). Descriptive statistics of all variables measuring the TPB constructs and risk perceptions are presented in Table 1. As reflected by mean values of item score measuring different constructs, participants exhibited the following: moderate-to-strong intention to use nanomaterials; somewhat positive attitudes; slightly positive perceived behavioral control and subjective norms; and moderate risk perceptions. Cronbach's alpha values ranged from 0.80 for perceived behavioral control to 0.93 for intention and attitudes, indicating high internal consistency.

Table 2 depicts sociodemographic characteristics stratified according to professional status. In line with the gender and professional distribution in the census of Norwegian dental health-care workers in the public dental healthcare service, 18.6% were male and 71.0% were dentists. The mean ± SD age of the participants was 41.5 ± 11.9 years. Of all respondents, 54.0% (63.7% dentists and 28.7% dental hygienists) confirmed that they had previously used dental nanomaterials.

Measurement model

Standardized factor loadings of all items were significant ($P < 0.001$) and ranged from 0.385 to 0.948 (results not shown). Standardized correlation coefficients ranged from 0.444 to 0.782 and were below the cut-off point of 0.85 (results not shown), indicating satisfactory discriminant validity of the latent constructs in the model [52].

The hypothesized correlated four-factor model approached acceptable fit, as indicated by fit indices (Table 3, Model 1). According to modification indices, the model fit could be improved by allowing correlation between residuals of items in the attitude construct (item 5 with item 6, item 7 with item 10) and in the subjective norms construct (item 21 with item 22) (Table 1). These residual correlations made theoretical sense and were therefore added to the model, one by one (Model 2 – Model 4). The final measurement model thus achieved a good fit (Table 3, Model 4).

Model 4 had an acceptable fit when applied separately for dentists ($\chi^2 = 522.9$; df = 200, $P < 0.001$, CFI = 0.947, RMSEA = 0.063 (90% CI = 0.057–0.070), SRMR = 0.048) and dental hygienists ($\chi^2 = 285.7$; df = 200, $P < 0.001$, CFI = 0.946, RMSEA = 0.058 (90% CI = 0.42–0.73), SRMR = 0.062). Configural invariance was supported as Model 4 fitted the data well across the two groups of dentists and dental hygienists ($\chi^2 = 788.4$ (df = 400), $P < 0.001$, CFI = 0.947, RMSEA = 0.062 (90% CI = 0.055–0.068), SRMR = 0.051). Metric invariance was also achieved as $\Delta\chi^2 = 0.655$ (df = 400–418, $P > 0.05$) and ΔCFI = 0.000.

Structural model

The full structural model had a good fit (Table 3, Model 5). All direct and indirect effects were in the expected direction. Within the model, all the hypothesized effects were

TABLE 2 Sociodemographic factors stratified according to professional status in the total sample

Factor	Dentist $n = 570$ % (n)	Dental hygienist $n = 228$ % (n)	Total $n = 798$[a], % (n)
Gender[**]			
Male	25.6 (139)	1.4 (3)	18.6 (142)
Female	74.4 (404)	98.6 (218)	81.4 (622)
Work experience[*]			
≤ 5 years	28.2 (161)	19.3 (44)	25.7 (205)
6–20 years	44.7 (255)	43.4 (99)	44.4 (354)
> 20 years	27.0 (154)	37.3 (85)	29.9 (239)
Place of education[**]			
Norwegian institution	68.7 (389)	96.5 (220)	76.7 (609)
Foreign institution	31.3 (177)	3.5 (8)	23.3 (185)
County region[ns]			
South-East	40.9 (233)	42.7 (97)	41.4 (330)
West	30.2 (172)	24.7 (56)	28.6 (228)
Middle-North	28.9 (165)	32.6 (74)	30.0 (239)
Past behavior[**]			
Yes	63.7 (311)	28.7 (54)	54 (365)
No/I don't know	36.3 (177)	71.3 (134)	46 (311)

[a]Number of participants is not 851 in each question because of missing values.
Testing the association between factor and professional status: [ns], not significant; *$P < 0.05$; **$P < 0.001$.

TABLE 3 Overall goodness-of-fit indices for the theory of planned behavior (TPB) measurement models (Models 1–4) and full structural model (Model 5)

Fit indices	Model 1	Model 2	Model 3	Model 4	Model 5
χ^2	782.3	680.6	612.8	555.9	665.5
df	203, $P < 0.001$	202, $P < 0.001$	201, $P < 0.001$	200, $P < 0.001$	236, $P < 0.001$
CFI	0.926	0.940	0.948	0.956	0.946
RMSEA	0.075	0.068	0.063	0.058	0.063
90% CI RMSEA	0.069–0.080	0.062–0.074	0.057–0.069	0.053–0.064	0.058–0.069
SRMR	0.049	0.048	0.045	0.042	0.045

Abbreviations: χ^2, chi-square test; df, degrees of freedom; CFI, comparative fit index; RMSEA, root mean square error of approximation; SRMR, standardized root mean square residual.

significant, except the direct effect of perceived risk on intention and indirect effect of perceived risk on intention through subjective norms (Table 4). Attitudes ($\beta = 0.53$, $P < 0.001$) and perceived behavioral control ($\beta = 0.24$, $P < 0.001$) were the strongest predictors of intention, followed in descending order by past behavior and subjective norms. Risk perception had a significant indirect effect on intention through attitudes and perceived behavioral control. Past behavior associated positively and directly with behavioral intention as well as indirectly through positive associations with attitudes, perceived behavioral control, and subjective norms. The total effect (indirect and direct) of risk perception on intention was negative ($\beta = -0.21$, $P < 0.001$), while the total effect of past behavior was positive ($\beta = 0.53$, $P < 0.001$). The augmented TPB explained, as expressed by R-squared, 74.5% of the variance in intention to use dental nanomaterials in comparison with the original TPB (attitudes, perceived behavioral control and subjective norms) that explained 71.8%. Multigroup analysis revealed that the fit of the model where regression paths were constrained was not significantly worse than the fit of the model where regression paths were free to vary ($\Delta\chi^2 = 0.32$; df = 490–501, $P > 0.05$; ΔCFI = 0.000). This confirms that regression paths were invariant across the two professional groups investigated.

DISCUSSION

The present study explains, using the TPB augmented with risk perception and past behavior, the intention of dental health-care workers to use nanomaterials in future treatment of patients. Although the direct effect of risk perception on

TABLE 4 Estimated standardized coefficients for the structural equation model (Model 5), showing the mediating effects between included variables

Direct effects	β	95% CI
Intention		
Attitudes (a)	0.53**	0.44 to 0.62
PBC (b)	0.24**	0.12 to 0.36
SN (c)	0.11*	0.04 to 0.18
Risk[a] (d)	0.00[ns]	−0.05 to 0.05
PB (e)	0.15**	0.09 to 0.21
Attitudes		
Risk (f)	−0.26**	−0.36 to −0.20
PB (i)	0.40**	0.34 to 0.47
PBC		
Risk (g)	−0.24**	−0.33 to −0.16
PB (j)	0.54**	0.48 to 0.59
SN		
Risk (h)	−0.03[ns]	−0.13 to 0.07
PB (k)	0.38**	0.31 to 0.44
Indirect effects		
a*f: Risk→Attitudes→Intention	−0.15**	−0.20 to −0.09
b*g: Risk→PBC→Intention	−0.06**	−0.09 to −0.02
c*h: Risk→SN→Intention	−0.01[ns]	−0.01 to 0.01
a*i: PB→Attitudes→Intention	0.21**	0.16 to 0.26
b*j: PB→PBC→Intention	0.13**	0.06 to 0.19
c*k: PB→SN→Intention	0.04*	0.02 to 0.07
Total effects		
Risk	−0.21**	−0.28 to −0.13
PB	0.53**	0.48 to 0.59

Abbreviations: β, standardized beta coefficient; PB, past behavior; PBC, perceived behavioral control; SN, subjective norms.

[a]'Risk' stands for 'Risk perception'.

[ns]Not significant.

*$P < 0.05$.

**$P < 0.001$.

intention was not confirmed in the hypothesized model, indirect effects of risk perception through attitudes and subjective norms were significant and in the expected direction. Thus, the findings confirm the structural validity of the hypothesized augmented TPB model, suggesting that this model is useful in identifying key socio-cognitive factors predicting the intention to use nanomaterials among dental health-care workers employed in the Norwegian public dental health-care service. Past behavior and risk perceptions added 2.7% to the explained variance in dental health-care workers' intention over and above that explained by the original TPB model (71.8%). The explained variance observed in this study compares with the data reported in some previous studies, whereby the TPB explained 65.0% of dentists' intention to apply fissure sealants, 69.0% of nurses' intention to recommend breastfeeding, and 77.0% of nurses' intention to accept information technologies [30, 31, 43].

One strength of the present study is the use of a census of dentists and dental hygienists working at public dental health-care service in Norway. Another strength is the use of a well-recognized theoretical framework, TPB, augmented according to the context with external variables. Moreover, structural equation modelling was employed to test the hypothesized model. This method is considered to be an advanced statistical technique that enables simultaneous testing of all relationships between both observed and latent variables in theoretical models, that would not be possible with ordinary regression analysis. Finally, high values of Cronbach's alpha indicated high internal consistency, suggesting that the items of the particular scales reflect the same underlying constructs. However, another reason for high coefficient value is the number of items measuring the construct. Specifically, attitudes were measured with nine items, which may result in an increased value of Cronbach's alpha [53].

Some limitations of this study should be addressed. Self-selection of the participants might have led to a selection bias if only those who were interested in the topic of nanotechnology or those who had some knowledge about nanomaterials replied, thus compromising the generalizability of the results. Moreover, the moderate response rate (47.5%) might also lead to limited generalizability. However, the gender and professional distribution of the respondents is consistent with that in the census of dental health-care professionals, supporting the external validity of the study. The cross-sectional nature of the data collection reflects the opinions of dental health-care workers at a particular time point, making it difficult to draw a conclusion about causal relationships. The present study did not assess actual behavior as the final outcome and in a prospective context as suggested by Ajzen [32]. Although intention is recognized to be a good proximal predictor of actual behavior, gaps between those constructs have been identified [26, 54]. Finally, the high percentage of explained variance observed in this study might reflect a problem of overfitting as a result of measuring all constructs at the same time and the problem of using self-reported data.

With regard to the relative importance of the three TPB constructs, attitude was the strongest predictor of intention to use nanomaterials followed by perceived behavioral control and normative pressure. Thus, the more favorably the use of nanomaterials was evaluated, the more confidence about managing such materials and the stronger the influence from immediate social environments, the stronger the intention among both dentists and dental hygienists. The importance of perceiving a relative advantage of using nanomaterials suggests that the decision of dental health-care workers was predominately considered as a personal choice. This finding contrasts with that reported in a review by Thompson Le-Duc [31], suggesting subjective norms to be the theory-based

construct most frequently associated with health professionals' shared decision-making behaviors. Also, in contrast with The findings of a systematic review by Godin et al. [30], which included analyses of various behaviors of health professionals suggested perceived behavioral control to be the most important predictor of behavioral intention, are also in contrast to the findings of the present study. Nevertheless, Perkins et al. [35], who also examined theory-based applications, concluded that the most important TPB construct varied across groups of clinicians and different behaviors. Consistent with the present study, attitudes have been identified as an important determinant of the intention of dentists to place fissure sealants in children's teeth [43], the intention of dental health-care workers to report suspected child maltreatment [33], and the delivery of preventive messages regarding diet, alcohol, and tobacco by dentists to their patients [36].

Perceived behavioral control played an important role in explaining intention in this study and was partly a reflection of past success and failures with the performances. This suggests that the perception of facilitating factors and barriers by dental health-care workers was influential. One plausible explanation might be that clinically related decisions, such as choosing nanomaterials instead of more conventional materials, is a complex procedure that requires various resources and is impacted by several aspects of the context, such as characteristics of the actual treatment and patients' acceptability or treatment preferences. Surprisingly, subjective norms were the weakest predictor of behavioral intention in this study, indicating that opinion of the immediate social environment was less influential regarding the use of dental nanomaterials. Even though dental health-care workers had, to some extent, experience with nanomaterials and past behavior had a positive effect on subjective norms, it is possible that the morals or principles of clinical behaviors reflecting professional norms regarding nanotechnology have yet to be established among Norwegian dental health-care workers. As stated by Ajzen, subjective norms present 'no clear pattern' [32]. Several systematic reviews have confirmed weak associations between subjective norms and behavioral intention [25, 55].

Incorporation of past behavior and risk perception into the TPB model increased the explained variance of the intended use of nanomaterials. This suggests that the three theoretical constructs of TPB did not provide an accurate description of the cognitions underlying the use of nanomaterials by dental health-care workers. Dental health-care workers who have already used nanomaterials would rather continue using them in the future. Moreover, previous experience had positive effects on the TPB constructs, suggesting that participants who had used nanomaterials possessed more positive attitudes, stronger perceived control, and higher perceived societal pressure. While some researchers criticize past behavior for not having predictive power, others, on the contrary, support inclusion of this factor in the model [41]. The present study is in line with the latter opinion, suggesting that past behavior had a significant effect on intention in the context of dental nanomaterials.

In contrast to the findings from the study by Zhu [29], there was no direct relationship between risk perception and intention, suggesting that this relationship is more complicated than originally hypothesized in this study. However, risk perception had an indirect effect on intention through attitudes and perceived behavioral control, indicating that participants with low perceptions of risk had more positive attitudes and stronger perception of control over their decision to use nanomaterials, which, in turn, were associated with higher intention to use such materials.

Prospective research should target participants from different countries to test the proposed model further. More studies are needed to uncover the relationship between the risk perception of dental nanomaterials and intention to use these nanomaterials. Apart from that, subsequent behavior should be assessed by using information from dental records instead of self-reports, as utilized in the present study.

In conclusion, the results of this study support the validity of the augmented TPB model to explain the intention of Norwegian dentists and dental hygienists to use nanomaterials. The strongest influence on intention is given by the attitudes toward nanomaterials and perceived confidence regarding their use. The findings of the study have implications for policy makers' management of the use of nanomaterials in dentistry.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Conceptualization: Marthinussen MC (equal), Cimpan MR (equal), Åstrøm AN (equal); **Investigation**: Xenaki V (equal), Åstrøm AN (equal); **Methodology**: Xenaki V (support), Marthinussen MC (support), Costea DE (support), Cimpan MR (support), Åstrøm AN (lead); **Formal analysis**: Xenaki

V (equal), Breivik K (equal), Lie SA (equal); **Writing – original draft**: Xenaki V (lead), Åstrøm AN (support); **Writing – review and editing**: Marthinussen MC (equal), Costea DE (equal), Breivik K (equal), Lie SA (equal), Cimpan MR (equal), Åstrøm AN (equal); **Supervision**: Marthinussen MC (support), Costea DE (support), Cimpan MR (support), Åstrøm AN (lead); **Funding acquisition**: Costea DE (equal), Cimpan MR (equal). All authors approved the final version of the manuscript for publication and agreed to be accountable for all the aspects of the work, ensuring that questions of accuracy or integrity of the work were appropriately investigated and resolved.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Paper III

Effect of TiO_2 nanoparticles on cell proliferation and viability: an *in vitro* study on 2D and 3D biological models

Xenaki V., Xue Y., Lie S. A., Marthinussen M. C., Åstrøm A. N., Costea D. E., Cimpan M. R.

Manuscript

III

9. Appendix

Questionaire in Norwegian

SPØRREUNDERSØKELSE

Bruk av odontologiske nanomaterialer:
tannhelsepersonellets oppfatninger om nanosikkerhet

Nanoteknologi er et raskt voksende vitenskapelig felt som er blitt en del av den moderne verden. Dette er et fagområde der det bevisst arbeides med strukturering og manipulering av materialer og systemer på nanometerskala (1 nm = 10^{-9} m). Partikler med minst en dimensjon som er mindre enn 100 nm blir kalt **nanopartikler.**

I dag inngår nanopartikler i mange hverdagsprodukter slik som kosmetikk og renholds midler samt medisinske og odontologiske materialer. Det er interessant at de samme egenskapene som gjør nanomaterialer gunstig også kan gjøre dem potensielt skadelige for miljøet og menneskers helse. Derfor har nanoteknologi reist mange etiske spørsmål som blant annet omfatter toksisitet (hvor giftige de er) og helsepåvirkning fra nanomaterialer.

Dette spørreskjema handler om bruk av nanoteknologi i tannhelsetjenesten. Det stilles spørsmål om din erfaring med, kunnskaper om- og dine holdninger til odontologiske nanomaterialer.

Nå kommer noen demografiske spørsmål.
Vi ber deg krysse av kun ett svaralternativ for hvert spørsmål

1. Jeg er (kjønn)

□ Mann
□ Kvinne

2. Hvor gammel er du? (alder)

_____

3. I hvilket fylke arbeider du? (fylke)

□ Østfold
□ Akershus
□ Oslo
□ Hedmark
□ Oppland
□ Buskerud
□ Vestfold
□ Telemark
□ Aust-Agder
□ Vest-Agder
□ Rogaland
□ Hordaland
□ Sogn og Fjordane
□ Møre og Romsdal
□ Sør-Trøndelag
□ Nord-Trøndelag
□ Nordland
□ Troms
□ Finnmark

4. Hva er din nåværende stilling i den offentlige tannhelsetjenesten? (stilling)

□ Tannpleier
□ Tannlege

5. Hvor mange år har du jobbet som tannlege/tannpleier? (årjobb)

□ Mindre enn 1 år
□ 1-5 år
□ 6-20 år
□ Mer enn 20 år

6. Hvor har du din grunnutdanning fra? (grunnutdann)

□ Universitetet i Oslo
□ Universitetet i Bergen
□ Universitetet i Tromsø
□ Høyskole i Norge
□ Institusjon utenfor Norge

Nå kommer noen spørsmål om hvor lett eller vanskelig det er for deg å forstå produktinformasjon som følger forpakninger av dentale materialer.
Vi ber deg krysse av kun ett svaralternativ for hvert spørsmål

1. Hvor ofte mener du det er vanskelig å forstå produktinformasjon som følger forpakninger av dentale materialer? (vanskforstaa)

□ Svært ofte
□ Ofte
□ Noen ganger
□ Sjelden
□ Svært sjelden
□ Aldri

2. Hvor ofte trenger du hjelp til å forstå produktinformasjon som følger forpakninger av dentale materialer? (hjelpforstaa)

□ Svært ofte
□ Ofte
□ Noen ganger
□ Sjelden
□ Svært sjelden
□ Aldri

Nå kommer noen spørsmål angående dine kunnskaper om bruk av dentale nanomaterialer i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hvert spørsmål

1. Hvor mye kunnskap har du om bruk av nanomaterialer i tannhelsetjenesten? (myekunnskap)

□ Ingen
□ Lite
□ Moderat
□ Mye
□ Veldig mye

2. Hvor mye informasjon har du fått om bruk av nanomaterialer i tannhelsetjenesten? (myeinfo)

□ Ingen*
□ Lite
□ Moderat
□ Mye
□ Veldig mye

*Dersom du har svart «ingen» gå til spørsmål **4**

3. Hvor har du fått informasjon om bruk av nanomaterialer i tannhelsetjenesten?

Kryss av alle svaralternativ som passer deg

Universitetet	□ ja	□ nei	**(hvoruniversitetet)**
Bøker	□ ja	□ nei	**(hvorbøker)**
Tidsskrift	□ ja	□ nei	**(hvortidsskrift)**
Aviser	□ ja	□ nei	**(hvoraviser)**
På internet	□ ja	□ nei	**(hvorinternet)**
TV	□ ja	□ nei	**(hvorTV)**

Radio	□ ja	□ nei	**(hvorradio)**
Kollega	□ ja	□ nei	**(hvorkollega)**
Salgsrepresentant for dentale materialer	□ ja	□ nei	**(hvorsalgsrepresentant)**
Andre	□ ja	□ nei	**(hvorandre)**

Nå kommer noen påstander om nanopartikler som vi ber deg vurdere som korrekt eventuelt ikke korrekt. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Nanopartikler er usynlige for det blotte øye (usynlige)
□ Korrekt
□ Ikke korrekt
□ Vet ikke

2. På grunn av liten størrelse kan nanopartikler penetrere cellene og vevene lettere sammenlignet med større partikler av det samme materiale (penetrere)

□ Korrekt
□ Ikke korrekt
□ Vet ikke

3. Nanopartikler kan være mer giftige sammenlignet med større partikler av det samme materiale (giftige)

□ Korrekt
□ Ikke korrekt
□ Vet ikke

4. Nanopartikler er allerede brukt i:

Tannkrem	□ ja	□ nei	□ Vet ikke	**(nanotannkrem)**
Munnskyll	□ ja	□ nei	□ Vet ikke	**(nanomunnskyll)**
Kompositter	□ ja	□ nei	□ Vet ikke	**(nanokompositt)**
Adhesiver	□ ja	□ nei	□ Vet ikke	**(nanoadhesiv)**
Rotfyllingsmaterialer	□ ja	□ nei	□ Vet ikke	**(nanorotfill)**
Avtrykksmaterialer	□ ja	□ nei	□ Vet ikke	**(nanoavtrykk)**

Nå kommer noen påstander om dine holdninger til bruk av nanomaterialer i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er en god ide (brukgodide)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

2. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er viktig (brukviktig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

3. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er farlig (brukfarlig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

4. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er forsvarlig (brukfosvarlig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

5. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er fornuftig med hensyn på kvaliteten på behandlingen (brukfornuftig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

6. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er uforsvarlig med tanke på pasientenes helse (brukuforsvarlig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

7. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er verdifullt (brukverdifullt)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

8. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er unyttig (brukunyttig)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

9. Å bruke dentale nanomaterialer ved pasientbehandling i fremtiden er interessant (brukinteressant)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

Nå kommer noen påstander om din hensikt eller plan om å bruke dentale nanomaterialer ved pasientbehandling i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Jeg har til hensikt å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (hensiktbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

2. Jeg planlegger å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (planbruk)

□ Helt enig
□ Ganske enig

□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

3. Jeg har bestemt meg for å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (bestemtbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

4. Hvor sannsynlig er det at du vil bruke dentale nanomaterialer ved pasientbehandling i fremtiden? (vilbruke)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

Nå kommer noen spørsmål angående dine erfaringer med bruk av nanomaterialer. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Har du brukt dentale nanomaterialer ved behandling av pasienter i tannhelsetjenesten? (harbrukt)

□ Ja*
□ Nei
□ Vet ikke

2.* *Hvis «JA», på spørsmål 1, *hvor ofte har du brukt dentale nanomaterialer ved behandling av pasienter i tannhelsetjenesten? (oftebrukt)

□ Svært ofte
□ Ofte
□ Av og til
□ Sjelden

Nå kommer noen påstander om hvor lett eller vanskelig det er for deg å bruke nanomaterialer ved pasientbehandling i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Dersom jeg vil har jeg muligheten til å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (mulighetbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

2. Det er helt og fullt opp til meg om jeg bruker dentale nanomaterialer ved pasientbehandling i fremtiden (opptilmegbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

3. Jeg har de ressurser jeg trenger for å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (ressurserbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

4. Jeg er sikker på at jeg er i stand til å bruke dentale nanomaterialer ved pasientbehandling i fremtiden (istandtilbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

5. Hvor lett eller vanskelig synes du det er å bruke dentale nanomaterialer ved pasientbehandling i fremtiden? (lettbruk)

□ Veldig lett
□ Ganske lett
□ Hverken lett eller vanskelig
□ Ganske vanskelig
□ Veldig vanskelig

Nå kommer noen påstander om hva andre mener om at du bruker nanomaterialer ved pasientbehandling i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Kolleger som har innflytelse på min kliniske praksis synes at jeg skal bruke dentale nanomaterialer ved pasientbehandling i fremtiden (kolleginnflyt)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

2. De fleste kollegaer som jeg kjenner vil bruke nanomaterialer ved pasientbehandling i fremtiden (flestekollegbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

3. Kolleger som er viktige for meg synes at jeg skal bruke dentale nanomaterialer ved pasientbehandling i fremtiden (kollegviktige)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

4. Min klinikksjef synes at jeg skal bruke dentale nanomaterialer ved pasientbehandling i fremtiden (klinikksjefsynesbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

5. Min klinikksjef vil selv bruke nanomaterialer ved pasientbehandling i fremtiden (klinikksjefbrukselv)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

6. Fylkestannlegen synes at jeg skal bruke dentale nanomaterialer ved pasientbehandling i fremtiden (ftsynesbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

Nå kommer noen spørsmål om opplevd risiko ved bruk av dentale nanomaterialer i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hvert spørsmål

1. Hvor sannsynlig tror du det er at du pådrar deg helseskade ved bruk av dentale nanomaterialer ved pasientbehandling i fremtiden? (selvhelseskade)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

2. Hvor sannsynlig tror du det er at du øker din egen risiko for å få kreft ved bruk av dentale nanomaterialer ved pasientbehandling i fremtiden? (kreftrisiko)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

3. Hvor sannsynlig tror du det er at du puster inn nanopartikler som akkumulerer i kroppen din dersom du bruker dentale nanomaterialer ved pasientbehandling i fremtiden? (pusterinn)

□ Svært sannsynlig
□ Ganske sannsynlig

□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

4. Hvor sannsynlig tror du det er at du medvirker til ukontrollert spredning av nanopartikler dersom du bruker dentale nanomaterialer ved pasientbehandling i fremtiden? (ukontrollspred)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

5. Hvor sannsynlig tror du det er at du påfører pasienten helseskader dersom du bruker dentale nanomaterialer ved pasientbehandling i fremtiden? (pasienthelseskade)
□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

6. Hvor sannsynlig tror du det er at du bidrar til miljøforurensing dersom du bruker dentale nanomaterialer ved pasientbehandling i fremtiden? (miljoforurensing)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

Nå kommer noen spørsmål som gjelder din eventuelle bekymring over bruk av dentale nanomaterialer.

1. Er du bekymret over økende bruk av dentale nanomaterialer ved pasientbehandling? (bekymretbruk)

Kryss av kun ett svaralternativ

□ Ja*
□ Nei
□ Vet ikke

2.* *Hvis «JA», på spørsmål 1, *hvorfor er du bekymret over økende bruk av dentale nanomaterialer ved pasientbehandling?

Kryss av alle svaralternativ som passer deg

På grunn av:

Helserisiko for pasient	□ ja	□ nei	**(bekymretpas)**
Helserisiko for tannlege	□ ja	□ nei	**(bekymrettannlege)**
Miljørisiko	□ ja	□ nei	**(bekymretmiljo)**
Uforutsette helseeffekter	□ ja	□ nei	**(bekymretuforutsett)**

Nå kommer noen påstander angående hvor trygt det er å bruke dentale nanomaterialer ved pasientbehandling. Vi ber deg krysse av kun ett svaralternativ for hver påstand

1. Det er trygt å bruke dentale nanomaterialer ved pasientbehandling (trygtbruk)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

2. Produsentene sørger for å minimere helserisiko forbundet med dentale nanomaterialer (produsentrisiko)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

3. Politikere sørger for å minimere helserisiko forbundet med dentale nanomaterialer (politikrisiko)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

Nå kommer noen spørsmål om dine interesser når det gjelder av dentale nanomaterialer i tannhelsetjenesten. Vi ber deg krysse av kun ett svaralternativ for hvert spørsmål

1. Hvor sannsynlig er det at du vil søke informasjon om bruk av dentale nanomaterialer? (searchinfo)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

2. Hvor sannsynlig er det at du vil følge nyheter om bruk av dentale nanomaterialer? (followneWs)

□ Svært sannsynlig
□ Ganske sannsynlig
□ Litt sannsynlig
□ Hverken sannsynlig eller usannsynlig
□ Litt usannsynlig
□ Ganske usannsynlig
□ Svært usannsynlig

3. Jeg ønsker å få mer informasjon om bruk av dentale nanomaterialer? (wantmoreinfo)

□ Helt enig
□ Ganske enig
□ Litt enig
□ Hverken enig eller uenig
□ Litt uenig
□ Ganske uenig
□ Svært uenig

Har du kommentarer til spørsmålene? (kommentarer)

Takk for at du deltok i denne spørreundersøkelsen!

Det vil bli trukket to I-pader blant de som har svart på spørreskjema!

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