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Reinforcement of Flowable Dental Composites with Titanium Dioxide Nanotubes

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Medical Biophysics

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REINFORCEMENT OF FLOWABLE DENTAL COMPOSITES
WITH TITANIUM DIOXIDE NANOTUBES

(Thesis format: Monograph)

by

Manal Dafar

Graduate Program in Medical Biophysics

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

Flowable composites are widely used in dentistry. However, they suffer from poor mechanical properties. The aim of this study was to reinforce a flowable composite with TiO₂ nanotubes (n-TiO₂), which were synthesized using an alkaline hydrothermal technique then functionalized with silane or methacrylic acid (MA). The synthesized n-TiO₂ was characterized using X-ray diffraction, energy-dispersive X-ray spectroscopy and Fourier transform infrared spectroscopy. Electron microscopy revealed a tubular morphology of n-TiO₂. Commercially available flowable composite was reinforced with varying amounts of nanotubes (0-5%). Dynamic Young's moduli (E) and fracture toughness (K_{IC}) of composites reinforced with 3% n-TiO₂ functionalized with MA exhibited the highest values. Cytotoxicity assays, performed on NIH/3T3 fibroblasts revealed excellent biocompatibility. We conclude that flowable composites reinforced with 3% n-TiO₂ exhibited superior mechanical properties to those of control, with a minimum effect on flowability and radiopacity. Thus, these reinforced composites represent promising materials for use in dental restorations.

Key words:

Flowable dental composites, TiO₂ nanotubes, flowability, dynamic Young's modulus, fracture toughness, radiopacity, MTT cytotoxicity.

Dedication

I dedicate my thesis to my loving parents, Osama and Salwa, my supporting husband, Faris, my precious daughters, Leen and Dana, my sisters, Amal and Walaa, and my brothers, Tamer, Ahmed and Rayan.

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List of Abbreviations and Symbols

Abbreviations:

% RGB:	percentage of red, green and blue
ADA:	American Dental Association
ATES:	allyltriethoxysilane
Bis-EMA:	ethoxylated bisphenol-A-dimethacrylate
Bis-GMA:	bisphenol A-glycidyl methacrylate
C-factor:	configuration factor
CF:	correction factor
CNSR:	chevron-notched short rod
D ₃ MA:	decanediol dimethacrylate
DMEM:	Dulbecco's Modified Eagle Medium
E:	Dynamic Young's modulus
EDX:	Energy dispersive x-ray spectroscopy
FBS:	Fetal bovine serum
FTIR:	Fourier transform infrared spectroscopy
GPS:	glycidoxypropyltrimethoxy-silane
ISO:	International Standards Organization
K _{IC} :	fracture toughness
LDH:	lactate dehydrogenase
MA:	methacrylic acid
MPS:	3-(trimethoxysilyl) propyl methacrylate
MPTMS:	3-methacryloxypropyltrimethoxysilane

MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
n-TiO ₂ :	titania nanotubes
NF n-TiO ₂ :	nonfunctionalized titania nanotubes
NTP:	notchless triangular prism
OD:	optical density of the X-ray image
OTMS:	trimethoxy (octyl) silane
PDF:	powder diffraction file
PMMA:	poly(methyl methacrylate)
SEM:	scanning electron microscopy
TEGDM:	triethyleneglycol dimethacrylate
TEM:	transmission electron microscopy
UDMA:	urethane dimethacrylate
UDMS:	urethane dimethacrylate silane
UTMA:	urethane tetramethacrylate
XRD:	X-ray diffraction

Symbols:

C_l :	longitudinal wave velocity (m/ μ s)
C_s :	shear wave velocity(m/ μ s)
$\text{\AA}$:	Angstroms
A:	the surface area of the fillers (m ² /g)
C :	velocity of sound (m/ μ s)
D:	specimen diameter (mm)

d :	specimen thickness (mm)
f :	the amount of fillers (g)
G' :	dynamic shear storage modulus (Pa)
G'' :	shear loss modulus (Pa)
I :	transmitted light
I_0 :	incident light
$P_{\max}$:	maximum load recorded during testing (N)
T :	total travelling time of the ultrasonic wave (μs)
$\tan \delta$:	loss tangent
W :	specimen length (mm)
X :	the amount of silane needed to coat the fillers (g)
γ :	shear strain rate
$\gamma^*_{\min}$:	the dimensionless stress intensity factor coefficient minimum
Δt :	the travelling time of the ultrasonic wave (μs)
η :	viscosity ($\text{N}\cdot\text{m}^{-2}\text{s}^{-1}$)
θ :	diffraction angle (degrees)
λ :	wavelength of the X-rays ($\text{\AA}$)
μ :	Dynamic shear modulus (GPa)
ν :	Poisson's ratio
ρ :	Density of specimens (kg/m^3)
τ :	shear stress (Pa)
ϕ :	fluidity ($\phi = 1/\eta$)
ω :	surface area coverage per gram of silane (m^2/g)

Chapter 1

Introduction

1.1 Historical background

Esthetic restorations that have the properties for matching natural color of teeth are in demand by patients and dentists. Thus, many trials have been done to find the best tooth colored restorations. The first attempt was using silicate cement as a white tooth filling material in 1873 by Thomas Fletcher [1]. These materials were supplied in a form of a powder and a liquid of alumino silicate glass and aqueous phosphoric acid respectively. The setting of this material occurred as a result of acid-base reaction that caused the material to be highly acidic. Due to its acidic nature, pulpal injury and dentin sensitivity were associated with silicate cements [2]. Resin-based dental materials were introduced at the end of the 1940's. Although these composites had much better esthetics than amalgam, the widely used restorative material at that time, their clinical use was not satisfactory. These early resins had very high polymerization shrinkage, low stiffness and unstable color. Subsequently, in 1951, Knock and Glenn [3] introduced a new material which had inorganic fillers incorporated with the resin to avoid polymerization shrinkage. However, discoloration and wear were problems encountered with these newer materials. Recently used dental composites were discovered in 1963 when Rafael Bowen discovered that chemical treatment of silica particles resulted in bonding with organic polymers. He used tris(2-methoxyethoxy) vinyl silane to coat the silica surfaces and referred to it as a coupling agent. Bowen was able to bind coated silica into bisphenol A-glycidyl methacrylate (bis-GMA) producing a material with very promising properties in the area of dental biomaterials [4]. This was a materials revolution in dentistry and the first stage in introducing dental composites – now so widely used in the practice of dentistry. Earlier, dental composites were composed of a continuous organic phase

formed by a fluid monomer and inorganic fillers of silica coated with a silane coupling agent. These composites were self-cured. They were prepared by mixing two pastes immediately before use. One paste contains an activator (e.g., tertiary amine) and the second paste contains an initiator (e.g., benzoyl peroxide) [5].

In the early 1970s, ultraviolet (UV) light-activated resin composites were introduced. It utilized UV light with an average wavelength 365 nm that can split benzoin methyl ether into free radicals (no tertiary amines) to start composite polymerization. Using UV light was not convenient because of its disadvantages including eye damage, possible soft tissue burns and shallow polymerization [6]. Within very few years Dart *et al.* [7] reported the use of visible light to cure composites by converting a photoinitiator into free radicals when exposed to the blue range wavelength. Flowable composites were introduced in 1996. Initially, the development of flowable composites was based on their higher fluidity but there was no clinical evidence of their success in clinical use. However, these composites were more subjected to wear due to their lower fillers content.

1.2 Composition of dental composites

Currently, dental composites consist of two phases: the synthetic polymer matrix and inorganic fillers. They are linked together via a coupling agent (such as silane). In addition, initiators and activators are added [8]. Bis-GMA is the most commonly used organic matrix in commercially available dental composites. Other monomers including triethyleneglycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), ethoxylated bisphenol-A-dimethacrylate (Bis-EMA), decanediol dimethacrylate (D₃MA),

bis(methacryloyloxymethyl) tricyclodecane, and urethane tetramethacrylate (UTMA) can be used to reduce the viscosity of Bis-GMA [9].

Inorganic fillers such as quartz, colloidal silica, and silica glass containing small amounts of zirconium, strontium and barium are the most commonly used fillers. The inorganic fillers play an important role in improving the strength and elastic stiffness. On the other hand, they reduce water absorption, polymerization shrinkage and coefficient of thermal expansion. Bonding of organic and inorganic phases by coating the fillers with a coupling agent results in the formation of a strong covalent bond. This is a key factor in terms of good mechanical properties of dental composite. Coupling agents have two functional groups in order to link the matrix and the fillers chemically [8]. Polymerization of light-activated composites takes place due to irradiation through blue light curing with the wavelength ranges between 410-500 nm. Photoinitiators are incorporated into dental composites to absorb that blue light [10]. Tertiary amine is used as a photoactivator [11]. Table 1.1 summarizes the composition of dental composites and the purpose of each ingredient [12].

Table 1.1: Chemical composition of resin dental materials		
Ingredient	Examples	Purpose
Principal monomer	Bis-GMA Bis-EMA UDMA	A primary monomer Forms polymer matrix Principal monomer
Diluent monomer	TEGDMA UDMA	Reduce the viscosity of the principal monomer
Inorganic fillers	Glass Ceramic	Provides strength Improves the optical properties
Silane coupling agent	γ methacryloxypropyl- trimethoxysilane	Bonds the filler to resin
Photoinitiators	Camphoquinone	Initiates polymerization reaction
Other chemicals for curing	Tertiary amine	Accelerate polymerization reaction
UV stabilizers	2-hydroxy-4-methoxybenzophenone	Prevents changes in shade
Inhibitors	Monomethyl ether of hydroquinone	Prevents self-polymerization
Radiopacifiers	Strontium, barium and lithium salts	Permit the material to be seen radiographically
Pigments and opacifiers	Iron and titanium oxides	Affect optical properties and shade match

1.3 Classification of dental composites

1.3.1 Filler size

According to the size of fillers, dental composites can be classified into macrofilled (conventional), microfilled, hybrid and nanofilled, Table 1.2 [5]. Composites with nanofillers are so called nanocomposites [8]. Different nanofillers have been introduced

to dental composites. However, in this thesis, we are introducing a novel flowable nanocomposites reinforced with TiO₂ nanotubes. Nanofillers do not scatter visible light as they shrink to a fraction of this light wavelength (0.4-0.8 μm). So, they are invisible and they can improve the optical properties of composites [13]. Theoretically, nanofiller content could be increased up to 90-95% by weight due to their small size. In fact, adding a high percentage of nanofillers affects their dispersion in the resin matrix and the viscosity of the nanocomposites because of their high surface area. Incorporating small weight fractions of nanofillers will result in significant reduction of polymerization shrinkage and further improvement in both physical and mechanical properties of nanocomposites [8].

Table 1.2: Classification of dental composites depending on the size of fillers

Type of composite	Size of filler (μm)	Type of filler	Advantages	Disadvantages
Macrofilled	10 – 40	Quartz or glass	Can withstand occlusal forces	1- Poor finish & high wear 2- Inadequate radiopacity of quartz
Microfilled	.01 – 0.1	Colloidal silica	1- Easier to polish. 2- Good surface finish	Lower mechanical properties than the macrofilled.
Hybrid	15 – 20 0.01 – 0.05	Glass and colloidal silica	Less polymerization shrinkage	Surface finish is less than microfilled
Modern hybrid	0.5 – 1 0.01 – 0.05	Glass, Zirconia and colloidal silica	Combine advantages of macro and microfilled	Less translucent
Nanofilled	< 0.01 (10 nm)	Silica or Zirconia	Improved strength and esthetic	Agglomeration of nanofillers

Studies reported that the smaller filler size has the ability to change the organic matrix between fillers resulting in better mechanical properties. This is due to reducing interparticle distances (at interfacial region) resulting in smooth transition between the stiff inorganic fillers to smooth organic polymer matrix [14, 15]. In contrast, nanofilled composites are characterized by larger interface between the small filler and organic matrix. Consequently, they could be more susceptible to hydrolytic degradation and a faster degradation of mechanical properties as compared to micro-hybrid composites [16].

Several types of dental nanocomposites are available commercially. Three examples are mentioned here. Filtek Supreme (3M ESPE, St. Paul, MN, USA) contains nanoclusters (silica or zirconia-silica particles, 2-75 nm) and nanomers (20-75 nm non-agglomerated, non-aggregated silica particles). Nanoclusters tend to form spherical agglomerates with average size of 0.6 μm . Another type of commercially available nanocomposite is Premise (Kerr/Sybron, Orange, CA, USA). It is a nanohybrid composite combining 3 different kinds of fillers: non-agglomerated 20 nm spherical silica nanoparticles, prepolymerized fillers (30-50 μm) and barium glass fillers (0.4 μm). The third example of nanocomposite is Ceram-X (Dentsply DeTrey, Konstanz, Germany). It contains glass fillers (1.1-1.5 μm), silicon-dioxide nanofiller (10 nm) and polysiloxane particles (2-3 nm) [8]. Table 1.3 summarizes these three nanocomposites.

Table 1.3: Summary of three commercial nanocomposites

Nanocomposite	Type of filler	Size of filler	Manufacturer
Filtek Supreme	1-Nanoclusters 2-Nanomers	2-75 nm 20-75 nm	3M ESPE, St. Paul, MN, USA
Premise	1-Nonagglomerated silica nanoparticles 2- Prepolymerized fillers 3- Barium glass fillers	20 nm 30-50 μm 0.4 μm	Kerr/Sybron, Orange, CA, USA
Ceram-X	1-Glass fillers 2-Silicon-dioxide nanofiller 3- Polysiloxane particles	1.1-1.5 μm 10 nm 2-3 nm	Dentsply DeTrey, Konstanz, Germany

1.3.2 Handling properties

Based on their handling properties, dental composites can be classified into universal, packable and flowable [12]. Universal composites can be used for almost all applications. Packable (condensable) composites are considered to be alternatives to dental amalgam. They are characterized by good physical and mechanical properties that enable them to withstand occlusal forces in posterior restorations.

Flowable composites are characterized by their greater fluidity due to either lower filler content or more diluent monomers. They offer the advantages of greater fluidity, easier placement and better adaptation to the walls of the cavity. They are indicated as liners, fissure sealants, preventive resin restorations (PRR), class V restorations and repair of fractured restorations [17]. Moreover, previous studies suggested the use of flowable composite with low modulus of elasticity as an intermediate layer under the hybrid composite. This may result in reducing the interfacial stress between the tooth and restoration [18]. However, the mechanical properties of flowable composites are inferior

to those of packable ones. Bayne *et al.* [19] reported that flowable composites have 20–25% lower filler content and subsequently lower rigidity and 20–30% lower modulus of elasticity [18]. Packable composites can be placed using a syringe or other dental instrument while flowable composites are formulated to be dispensed from a syringe with very fine disposable tips [11].

1.4 Clinical failure of dental composites

Researchers are trying to understand the factors leading to failure of composite restorations. Recurrent caries found to be the most common cause of failure especially with posterior restorations [20]. However, other studies showed evidence for restoration failure after 5 years due to bulk fracture [21].

1.5 Composite reinforcement trials

With respect to the inferior mechanical properties of flowable composites, many attempts were made to reinforce resin-based composites. Fiber reinforcements with ceramic and metal fibers are the most common approaches to improve the mechanical properties of dental and orthopedic composites [22, 23]. The success of such reinforcements was not ideal due to the large fillers size, poor filler-matrix bonding, unequal distribution of fillers, and the presence of stress concentration areas [24]. Until now, no fillers are defined as ideal for dental composites. However, TiO₂ nanotubes incorporated into flowable resin composites could improve the fracture and flexural properties without significant alteration in their fluidity [25].

1.6 Nanofiller structures and fabrication methods

Nanofiller morphology varies depending on the preparation method. Different nanostructure morphology may influence their reinforcing effect on composites. Ding *et al.* [26] defined four different groups of nanostructures. *Nanowires* are linear structures, but their sides or cross sections may not be uniform; *nanorods* are similar to nanowires, but shorter; *nanoribbons/nanobelts* have well-defined and uniform walls; and *nanotubes* have a characteristic hollow interior channel (Fig. 1.1).

Fig.1.1: Nanostructure morphologies; nanowires, nanorods, nanotubes, and nanobelts. Adapted with permission (Appendix A) from the journal of physical chemistry B. 2004;108(33): 12280-91 [26].

Titania nanotubes (n-TiO₂) can be fabricated using several techniques, including the assisted-template method [27], sol-gel method [28], electrochemical anodic oxidation method [29] and hydrothermal treatment [30]. Ou *et al.* [31] compared different n-TiO₂

preparation techniques (Table 1.4). In the work presented in this thesis, we used the alkaline hydrothermal method at 160°C followed by washing with deionized water and 0.1 N HCl to fabricate n-TiO₂.

Table 1.4: Comparison between different n-TiO₂ fabrication techniques			
Fabrication method	Advantages	Disadvantages	n-TiO₂ features
Template-assisted method	1- The scale of nanotube can be moderately controlled by applied template	1- Complicated fabrication process 2- Tube morphology may be destroyed during fabrication process.	Ordered arrays (powder form)
Electrochemical anodic oxidation method	1-More desirable for practical applications 2-Ordered alignment with high aspect ratio 3-Feasible for extensive applications	1-Mass production is limited 2-Rapid formation kinetics is subjected to the utilization of HF 3-Highly expense of fabrication apparatus	Oriented arrays (thin film)
Hydrothermal treatment	1-Easy route to obtain nanotube morphology 2-A number of modifications can be used to enhance the attributes of titanium nanotubes 3-Feasible for extensive applications	1-Long reaction duration is needed 2-Highly concentrated NaOH must be added. 3-Difficult in achieving uniform size	Random alignment (powder form)

1.7 Biomedical applications of titania nanofillers

n-TiO₂ have been found to have excellent biocompatibility [25]. As reported by Swami *et al.* [32], n-TiO₂ with modified surfaces have been used in orthopedics and dental implants. Also, n-TiO₂ were a suitable substrate for bone regeneration using stem cells. In addition, these nanotubes could be used as drug delivery vehicles for local antibiotics.

Yoshikawa *et al.* [33] reported that calcium phosphate cement containing anatase phase of TiO_2 contributes to apatite formation *in vitro*. This led to the application of titania-based cements in endodontics as root canal sealers. These cements exhibited excellent sealing ability and controlled setting times [33]. n- TiO_2 were successfully incorporated into resin-based bone cements as reinforcing fillers [25].

1.8 Nanofiller surface modifications (functionalization)

Functionalization can be defined as the chemical reaction to treat/modify the inorganic fillers surface in order to be compatible with the organic polymer matrix of the composite. Surface modification of n- TiO_2 particles is required because these nanoparticles are very fine and can easily agglomerate. On the other hand, they are difficult to disperse in organic solvents. Organosilanes are commonly used to coat inorganic fillers of dental materials to improve their dispersion and bonding to the resin matrix [34].

For functionalization with silane, two main methods have been proposed. Hydrolysis and direct condensation mechanisms. As described by Pluedeman [35], hydrolysis mechanism involves hydrolysis of ($-\text{OCH}_3$) groups on silane results in formation of silanol groups. The silanol groups condense with hydroxyl groups on the surface of inorganic fillers to form a covalent bond. This method needs to be done in a water-containing organic solvent such as cyclohexane. The second mechanism is direct condensation in which silane binds to the filler surface chemically through direct condensation of alkyloxy groups of the silane with hydroxyl groups on the fillers surface. This method is carried out in an anhydrous environment [36]. Amines can also be used as

catalysts to promote adsorption of silane. According to Chen *et al.* [37], 2% n-propylamine used with 3-methacryloxypropyltrimethoxysilane (MPS) forms a more stable silane bond.

The interphase between organic matrix and fillers has a significant role in nanocomposites as the nanosized fillers have high surface area to volume ratio. Wilson *et al.* [38] studied the effect of organosilanes on nanocomposites. Two types of silane were used, a reactive and non-reactive silanes (dual silanization). The reactive silane; 3-methacryloxypropyltrimethoxysilane (MPTMS) has the ability to copolymerize with the polymer network while the nonreactive silane; n-octyltrimethoxysilane (OTMS) does not copolymerize but it is hydrophobic which may prevent water sorption and hydrolytic degradation of composite. Methacrylic acid (MA) was found to be able to bind with n-TiO₂ via a Ti-carboxylic coordination bond. Also, copolymerization of the vinyl double bond in MA results in formation of nTiO₂-PMMA composite [39]. Nanosized TiO₂ reinforcing fillers were introduced to dental composites by Xia *et al.* [40] in form of nanoparticles. Allyltriethoxysilane, (ATES) was used for functionalization of these nanoparticles. ATES functionalized nanoparticles have a limited improvement in the mechanical properties of dental composites because of their agglomeration.

1.9 Characterization of nanotubes

1.9.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) is used to characterize the structure and composition of materials. Each material has its own XRD pattern caused by diffraction of an incident X-ray beam when it interferes with the atomic planes of a crystal. XRD can be used to

identify crystal structures of elements or to identify unknown materials or mixtures by comparing the experimental data with databases [41]. XRD pattern for TiO₂ varies according to its different crystal structures. Each pattern results due to interaction between the incident X-rays and the sample creating diffraction beams. These diffracted beams are related to interplanar spacing (d-space) in the crystalline powder. According to Bragg's Law: $\lambda = 2d \sin\theta$ where λ is the wavelength of the X-rays (Å), d is the interplanar spacing (Å) and θ is the diffraction angle (degrees). In case of Cu K α , $\lambda=1.54$ (Å). Lin *et al.* [42] reported diffraction peaks for anatase TiO₂ matched with Powder Diffraction File (PDF#21-1272) in databases.

1.9.2 Energy dispersive x-ray spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy (EDX) is a common analytical technique used for elements analysis. It depends on the interaction between X-rays and a sample. Each element has a characteristic atomic structure that results in a unique set of peaks. To stimulate X-rays emission from a sample, a high-energy beam with charged particles (either electrons or protons) or X-ray beam is directed to the sample of interest. This beam leads to electron excitation in the inner shell of the sample's atoms followed by electron ejection leaving a hole in the inner shell. This hole will be filled with an electron from the outer shell of the atom. The difference in energy between the inner and outer shells may be released as X-ray that can be measured by an energy-dispersive spectrometer [43].

1.9.3 Fourier transform infrared spectroscopy (FTIR)

To confirm bonding between n-TiO₂ and coupling agents via different functional groups,

Fourier transform infrared spectroscopy (FTIR) can be done. FTIR can be used to determine the presence of functional groups in molecules by comparing the experimental FTIR absorbance or transmission peaks with databases. When new fillers are introduced to composite, FTIR is a valuable method in evaluating the functional groups that bind the fillers with the polymer matrix. In the literature, many studies were conducted to understand bonding between functionalized fillers and resin matrix. Yang *et al.* [44] studied the bonds formed between TiO₂ particles and poly (methyl methacrylate) (PMMA). FTIR showed an evidence of presence of vinyl groups causing PMMA grafting from the surface of TiO₂ particles. FTIR absorption band at 1700 cm⁻¹ corresponds to carbonyl group stretching in silane confirming that vinyl groups were successfully grafted into TiO₂ surfaces. Khaled *et al.* [25] reported FTIR absorbance peaks between 1413-1547 cm⁻¹ due to the presence of carboxylic group and another peak at 1630 cm⁻¹ due to coordination of vinyl bond of MA with TiO₂ nanotubes.

1.10 Flowability of dental composites

Improving the strength of flowable composites was the main target of this study. However, maintaining their fluidity was essential. It is important here to clarify that flow is the fluidity (ϕ) of the material and it is the reciprocal of viscosity ($\phi = 1/\eta$). Flow is a function of shear rate and temperature. It is a term used to describe how quickly materials flow in a certain period of time, whereas viscosity is the material's resistance to flow. Viscosity (η) is the shear stress (τ) divided by the shear strain rate (γ) under the same temperature. Dental composites are viscoelastic materials. They share criteria of both viscous materials (e.g., oils) and elastic materials (e.g., metals).

The dynamic oscillatory shear test is commonly used to study the viscoelastic behavior of a material. Lee *et al.* [45] investigated the viscoelastic properties of different composites including flowable ones. A vibratory shear signal of frequency (ω) was applied to the composite specimens. Afterwards, the dynamic shear storage modulus (G'), the shear loss modulus (G''), the loss tangent ($\tan \delta$) and the viscosity (η) were measured. Evaluation of flowability can be done by using different methods other than those that are used to evaluate viscosity. Bayne *et al.* [19] compared the flowability of different commercial flowable composites. They combined the American Dental Association (ADA) flow test for dental cements [46] with a suggested flow test for composites [19, 47]. A standard volume of material was dispensed and sandwiched between two glass slides and permitted to flow for 30 s then light cured. The diameters of resulted composite discs were measured and compared. A similar technique was used by Attar *et al.* [17] who also compared the flowability of different composites. In the study reported in this thesis, we modified the ADA flow test for dental cements and combined it with previously published methods to evaluate the flowability.

1.11 Mechanical properties of dental composites

1.11.1 Factors affecting mechanical properties

Mechanical properties of composites are greatly affected by inorganic fillers. There is a direct relation between filler content and mechanical properties. In terms of modulus of elasticity, the higher the filler content, the higher the modulus and subsequently greater deformation resistance [48]. On the other hand, filler content is inversely proportional with polymerization shrinkage. Highly filled composites associated with less volumetric

shrinkage [49]. Stress development in composite restorations is influenced by the material's stiffness and flowability, both of them are affected by the fillers type, size, shape and their surface treatment [50]. Ilie *et al.* [51] reported that filler weight % has the strongest influence on the mechanical properties of composites while filler volume's effect was less pronounced. However, low correlation was found between filler weight and fracture toughness. On the other hand, matrix-filler interaction in composites seems to play an important role in increasing the material's toughness especially with smaller filler size such as in nanohybrid composites.

The microstructure of nanocomposites has a great influence on their mechanical properties. Nanoparticle volume fraction, aspect ratio and the interface between nanoparticle and matrix affect the composite behavior. The resin matrix molecular structure could be altered at the interface due to interaction with nanoparticles [52]. The C-factor (configuration factor) of the cavity is defined as the ratio of the bonded to unbonded surfaces of the filling. It is an essential factor that contributes to stress contraction and gap formation in composite restorations [53, 54]. Placing the restoration in an incremental technique is a common clinical approach to minimize the contraction stress associated with C-factor. Furthermore, applying a flowable composite lining underneath the main filling could be effective in reducing shrinkage stress [55].

1.11.2 Dynamic Young's modulus (E)

Dynamic Young's modulus is a term that describes a material's stiffness. Along with the adhesive properties, elastic modulus is an important factor in terms of microleakage, recurrent caries and dislodgement of restoration [56]. In other words, elastic modulus has

a direct relationship with a restorative material's reliability [57]. Also, higher filler content would improve strength, hardness and elastic moduli of resin materials [58].

Materials with different elastic moduli are required for different dental applications. Composites with low elastic moduli are required for class V restorations to allow their flexure with the tooth. In contrast, composites with higher elastic moduli are more suitable for class I and class II restorations in order to withstand higher occlusal forces and to avoid deformation and cusp fracture [56].

Few methods have been described for measuring dynamic and static elastic moduli. Three-point bending test [56, 59] and tension [56, 60] are the most common methods for static moduli measurements. However, dynamic mechanical thermal analysis (DMTA) [61] and ultrasonic pulse method [62] are used to measure the dynamic modulus of elasticity.

In this thesis, we used a nondestructive ultrasonic pulse method to measure the dynamic Young's moduli. Measuring dynamic elastic properties gives an idea about the micro-structural rigidity and the extent of inter-phase adhesion within the composite materials [62]. The ultrasonic waves have the ability to propagate in elastic bodies by means of vibration or oscillation of their particles. This oscillatory motion could be in longitudinal or transverse directions. The main idea of this technique is to analyze the time required for ultrasonic wave propagation by using following components; a pulse function generator, transmitting and receiving piezoelectric crystals (lithium niobate crystals), amplifier, and oscilloscope. The pulse generator applies a high-frequency pulse that causes an ultrasonic mechanical vibration to be emitted by piezoelectric transducers. These transducers convert the electrical pulses into mechanical vibration. This

piezoelectric effect is reversible. As the ultrasonic wave reaches the piezoelectric element, it also generates a similar frequency pulse at its electrodes, which can be delivered and recorded on the oscilloscope screen. Therefore, two lithium niobate crystals were attached to the two parallel surfaces of the specimens. One crystal emits an ultrasonic wave through the material while the other crystal receives the wave and converts it into an electrical signal. Both the transmitting and receiving signals were recorded in oscilloscope and the wave's travel time was determined from the receiving signal recorded in the oscilloscope. The ultrasonic pulse method is considered to be one of the most convenient techniques in evaluating the materials elastic properties. This technique has the following advantages: it can be used to measure the material's rigidity and it gives a qualitative reflection of the interfacial adhesion between the reinforcing fillers and matrix of the composite [62]. Furthermore, this method has a very low coefficient of variation [63] and is therefore a very valuable for testing new materials.

1.11.3 Fracture toughness (K_{IC})

Fracture toughness is defined as the energy absorbed by the material prior to crack propagation. In other word, it is a measurement of the amount of energy required for fracture. It is a standard test used for studying fracture behavior of orthopedic and dental composite materials. Fracture due to crack propagation occurs in three different loading conditions; modes I, II and III [64] (Fig. 1.2). In mode I (opening mode), crack propagates under tension due to pulling its surfaces apart. In mode II (sliding mode), the body is loaded by shear forces perpendicular to the crack front and parallel to the crack surfaces causing their sliding over each other. Mode III (tearing mode) occurs when the

body is loaded by shear forces parallel to the crack front, causing sliding of the crack surfaces [65].

Fig 1.2: Fracture modes due to crack propagation. (a) mode I, (b) mode II and (c) mode III. Reproduced with permission (Appendix A) from the journal of dental materials 2010;26(2):63-77 [64].

Many studies have been conducted to understand the fracture mechanics. Researchers suggested that under stress conditions, failure starts from the highly stressed peripheral areas of the bonded surfaces towards the center [64, 66]. Griffith *et al.* [67] studied the mechanism of composite restorations failure. Their results are in line with the previous ones. They concluded that polymerization shrinkage takes place after curing of composite. This shrinkage pulls the walls of the cavity towards the center.

Different testing methods have been described for K_{IC} measurement. Fracture toughness is an intrinsic property of materials. Therefore, its values should be independent on the measurement method. However, different K_{IC} values were reported for same materials measured with different methods. This variation could be related either to difficulties in producing appropriate specimens or difficulties in conducting the test [64, 68].

Fujishima and Ferracane [68] compared the K_{IC} values of dental composites using

four different methods including single-edge notched, compact tension, chevron-notched short rod (CNSR) and double torsion. In the single-edge notched method, a notched bar specimen was subjected to three-point bending. The compact tension method applies load to a notched plate in uniaxial tension. A cylinder with a chevron-shaped notch is loaded in tension in the CNSR method. The specimen preparation for this method was difficult as it requires cutting a chevron notch which is hard to control especially with small samples [69]. In the double torsion method, a notched and grooved plate was loaded in four-point bending.

Both the single-edge notched and the compact tension methods were described for metallic materials. They require crack propagation from a fatigue produced pre-crack which is difficult in dental composite specimens because of their small size. Thus, these two methods are less commonly used for composites. In contrast, the double torsion method does not require a pre-crack to initiate the fracture. Instead, the first crack produced in the test causes propagation of subsequent cracks. Similarly, the CNSR test produces a stable crack region, which acts as a pre-crack for crack propagation. It is one of the most common methods for testing K_{IC} of dental composites [70, 71].

Among these four tests, the double torsion test was the most difficult one because of its demanding requirements in preparation and alignment of the samples. Specimen failure in this test was associated with fracture out of the crack's plane. Similarly, failure of the CNSR specimens was also due to deviation of the crack fracture plane from the plane of the notch. However, the single-edge notched and compact tension methods showed flat fracture planes with no evidence of deviation. [68]

Ruse *et al.* [69] described the use of notchless triangular prism (NTP) for K_{IC}

measurement to overcome the difficulties associated with CNSR method. The NTP specimen ($6 \times 6 \times 6 \times 12$ mm) was fitted in a holder and secured with screws. A crack (0.1 mm in depth) was created. The holder including the specimen was then mounted on an Instron machine and tested at a crosshead speed of 0.1 mm/min under tension until fracture (Fig. 1.3, 1.4). The maximum load at fracture and displacement were recorded.

Earlier studies of fracture toughness suggested that crack freezing could occur in dental composites when crack is stopped from propagation due to fillers or molecular chains [72]. Other investigators proposed crack bowing effect as a result of pinning by the fillers. Other researchers reported crack branching due to increasing the crack surface area with increasing the filler content [73].

Fig. 1.3: (a) Notchless triangular prism (NTP) used for fracture toughness test. (b) Diagram NTP specimen mounted in holder. Adapted with permission (Appendix A) from the J Biomed Mater Res. 1996; 31(4): 457-63 [69].

Fig. 1.4: (a) Holder for the NTP specimen, (b) Mounting block with specimen holder, (c) Mounting block with specimen holder, NTP sample, and spacer blade, and (d) Custom-designed grips securing the holder and an NTP specimen. Adapted with permission (Appendix A) from the J Biomed Mater Res. 1996;31(4):457-63[69].

1.12 Radiopacity of dental composites

Radiopacity of dental restorations is a critical parameter for accurate diagnosis and treatment planning. It is crucial to use materials with adequate radiopacity in order to distinguish them from the natural tooth structures. Moreover, dental restorations should be radiopaque enough to detect recurrent caries, overhanging margins, proximal contacts and restoration contour [74]. As specified by the International Standards Organization (ISO 4049), the radiopacity of dental materials should be equal to or greater than the same thickness of aluminum wedge and should not be less than 0.5 mm of any value claimed by the manufacturer [75]. Radiopacity of composite materials should exceed that of dentin. However, no definitive maximum limit has been identified.

Radiopacity is a quantitative measure for dental restorations against a standard dental X-ray source. Three methods are known to measure the radiopacity of dental materials including transmission densitometry, direct digital radiography, or indirect

digital radiography. Measuring the optical density (OD) of the X-ray image with a densitometer is a common technique for evaluating radiopacity. OD is calculated as a logarithm of the ratio of light transmitted through the film to the incident light according to the following formula: $OD = \log_{10} (I/I_0)$ where (I) is transmitted light and (I_0) is incident light. The OD is correlated with the absorption of X-rays by the radiopacifiers. Higher OD associated with lower radiopacity [76, 77]. Direct digital method utilizes certain image sensor to provide a direct electronic reading. On the other hand, indirect digital method works by digitizing chemically processed radiographs followed by the use of specific digital color meter to identify the grey values [78]. In the work presented in this thesis, we used the indirect digital method to evaluate the radiopacity of n-TiO₂ reinforced composites.

1.13 Cytotoxicity

All synthetic materials inserted in the human body must be tested for biocompatibility. Biocompatibility is the material's ability to function in a host tissue without causing any adverse reaction [79]. Cytotoxicity describes the cascade of molecular events that causes either cellular or functional damage [80]. In case of dental restorations, adverse reactions could be dentin sensitivity, gingival inflammation or loss of pulp vitality.

Previous studies have argued that dental composite resins cause adverse reactions. These studies reported that un-polymerized monomers might leach into saliva [81, 82]. Residual monomers or polymerization products could be released after composite setting into the oral cavity or adjacent tissues [83, 84]. Imazato *et al.* [85] reported that 20-25% of monomers remained unreacted after polymerization. Given the fact that flowable

composites have more monomers and less filler content as compared to hybrid ones, more leaching components could be associated with these composites [83].

The International Organization for Standardization (ISO) guidelines for testing new materials include (ISO 7405: Preclinical evaluation of biocompatibility of medical devices used in Dentistry) [86] and (ISO 10993: Biological evaluation of medical devices) [87]. These guidelines recommend standard practices for evaluating dental materials biologically including the following. (i) It is incumbent upon the dental material manufacturer to select the appropriate tests, based on the intended use of the material, and known and assumed toxicity profile of the material or its components. (ii) A manufacturer may select one of three cytotoxicity tests in preference to another because of cost, experience or other reasons. (iii) Overall, there are four levels of testing. New materials should be evaluated using initial cytotoxicity and secondary tissue screening tests prior to extensive animal testing and clinical trials. (iv) The test result should always be evaluated and interpreted with consideration for the manufacturer's stated use for the material.

Fibroblasts are the most commonly used cells in testing the biocompatibility of new dental materials [88]. They can be isolated from patients easily and they can grow in regular culture media, although cell lines such as L-929 and 3T3 mouse fibroblasts are used frequently [79]. Many *in vitro* tests can be conducted to evaluate the cytotoxicity. However, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was found to be one of the most sensitive [89]. It is the most commonly used test to evaluate dental material cytotoxicity because it is an easy, fast and inexpensive test [82, 90, 91]. The MTT assay was described by Mossmann (1983) in order to detect the

viability and proliferation of mammalian cells [92]. It depends on MTT yellow tetrazolium salt cleavage to purple formazan crystals utilizing mitochondrial dehydrogenase in metabolic active cells. The formed purple formazan crystals accumulate in healthy cells as they are insoluble and do not cross the cell membrane. The optical absorbance is directly proportional to the amount of dissolved formazan and the number of viable cells [93]. Issa *et al.* [90] tested different dental monomers and their toxic effect on human gingival fibroblast using two different cytotoxicity tests. MTT was used to measure fibroblasts metabolism and the cytosolic enzyme lactate dehydrogenase (LDH) was used as a measure of cell integrity. The effects of tested monomers on gingival fibroblast mitochondrial activity found to be reproducible and dose dependent when tested with MTT.

1.14 Thesis motivation, objectives and hypothesis

The demand for flowable dental composites with reasonable mechanical properties, excellent flowability and easy handling properties motivated us to work on this project. From the literature, n-TiO₂ were identified as promising reinforcing fillers for composites. The objectives of this study were to: (i) introduce n-TiO₂ as reinforcing fillers into a flowable dental composite, (ii) evaluate the effect of n-TiO₂ content on flowability, (iii) measure the dynamic Young's modulus (E) and fracture toughness (K_{IC}) of the control and reinforced composites, and (iv) evaluate radiopacity and potential cytotoxicity *in vitro*. We hypothesized that using n-TiO₂ as reinforcing fillers in flowable dental composites would significantly improve their mechanical properties without altering their flowability.

Chapter 2

Materials and Methods

2.1 Chapter outline

In this chapter, we describe the experimental setup used for the synthesis of n-TiO₂, their functionalization and chemical characterization. Also explained are the preparation of reinforced flowable composites, assessment of their flowability, and testing of their mechanical properties, radiopacity and cytotoxicity.

The synthesis of n-TiO₂ took place using an autoclave bomb. Functionalization of the synthesized n-TiO₂ was done either by using a rotary evaporator for silane functionalization or by using a reflux condenser for MA functionalization. The reinforced flowable composite samples were prepared by manual incorporation of functionalized n-TiO₂ into commercially available flowable composites followed by light curing according to standard clinical procedures. A few methods of characterization were employed to examine the synthesized and functionalized n-TiO₂ including X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The Dynamic Young's moduli (E) and fracture toughness (K_{IC}) of composite specimens were measured.

2.2 Nanotube synthesis

Synthesis of n-TiO₂ was carried out in a Teflon-lined stainless steel autoclave (a 125 mL acid digestion bomb (Parr 4748, Parr Instrument Company, Illinois, USA). The temperature was controlled using a T-thermocouple and a temperature controller (Omega HRC5A). Samples of n-TiO₂ were synthesized using the alkaline hydrothermal technique described earlier by Kasuga *et al.* [28]. Basically, the experiment was started by

dispersing 0.24 g strontium acetate powder in 30 mL of 10 M NaOH solution and stirring for 24 h, followed by addition of 2 g of commercially available TiO₂ nanopowder and stirring for another 24 h. The mixture was transferred to a Teflon-lined stainless steel autoclave for hydrothermal treatment. This reaction was maintained at 160°C for 20 h. Afterwards, the reaction product was centrifuged and washed with deionized water until the pH dropped to 10.0 then washed with 0.1 N HCl until the pH dropped to 6.0 at room temperature. The resulting product was dried in a vacuum oven at 80°C overnight and then calcined at 400°C for 2 h.

2.3 Nanotube functionalization

2.3.1 Silane functionalization

This reaction was started by dispersing the n-TiO₂ in 20 mL cyclohexane and 0.028 mL n-propylamine. The silane mixture of 3-(trimethoxysilyl) propyl methacrylate (MPS) and trimethoxy (octyl) silane (OTMS) was added (7.5% MPS & 2.5% OTMS). The reacting materials were kept under constant stirring at room temperature for 24 h then at 60°C for 30 min. The mixture was placed in a rotary evaporator and the temperature elevated to 95°C for 1 h to evaporate the solvent. Afterwards, the reaction product was subjected to vacuum drying at 80°C overnight. Earlier studies suggested that using 10% silane is more than enough to coat the filler surfaces completely. The amount of silane required to coat the filler was calculated using the following equation [38]: $X = (A/\omega)f$, where X is the amount of silane (g) needed to coat the fillers, A is the surface area of the filler (m²/g), ω is the surface area coverage per gram of silane (m²/g), and f is the amount of filler (g). In the case of n-TiO₂ used in this study, the surface area $A = 330$ m²/g and the surface area

coverage per gram of silane $\omega = 2525 \text{ m}^2/\text{g}$ [38]. Therefore, the amount of silane used to coat 1 g of n-TiO₂ in this work was 0.13 g. As we used dual-silanization with 10% silanes, we mixed 7.5% MPS with 2.5% OTMS.

2.3.2 Methacrylic acid (MA) functionalization

The MA functionalization reaction was carried out under a reflux condenser at 85°C for 24 h. The reaction was started by dispersing 0.1 g n-TiO₂ in 35 mL of 2-propanol then 3 mL MA was added. The pH was adjusted to 5.0 by adding drops of 0.3 N KOH. The reaction product was subjected to vacuum drying at 80°C overnight [25].

2.4 Characterization of n-TiO₂

2.4.1 X-ray diffraction (XRD)

XRD was carried out using CuK α radiation (30 kV, 10 mA) in order to examine the n-TiO₂ crystalline phase following the alkaline hydrothermal method and calcination at 400°C. The samples were prepared for XRD by gentle grinding 0.1 g of the calcined powder using a mortar and pestle. The powder was placed evenly on a plastic XRD flat plate and placed in the XRD machine. Data were collected at a scattering angle of 2θ from 2° to 82° with 0.05° step size and 10°/min scan speed (BRUKER AXS D2 phaser, Karlsruhe, Germany). 2θ for equivalent CuK α radiation was calculated using Bragg's Law [94].

2.4.2 Energy dispersive X-ray spectroscopy (EDX)

The atomic proportions of Ti, O and Sr atoms in n-TiO₂ were determined using FIB/SEM

LEO 1540XB microscope equipped with an EDX spectrometer (Carl Zeiss, Jena, Germany).

2.4.3 Fourier transform infrared spectroscopy (FTIR)

TiO₂ functionalized surfaces were investigated by FTIR using the KBr pellet method (Bruker Vector 22 FT-IR Spectrometer, Bruker Optic Inc., Ettlingen, Germany). Sample preparation was done by adding 0.2 g anhydrous KBr to 2 mg of either silane or MA functionalized n-TiO₂ powder (after gentle grinding with mortar and pestle). The prepared powder was pressed at 10 tons for 10 min. using stainless steel die and bolts. Finally, the sample was placed into a sample holder for analysis. Data were collected at wavenumbers ranging between 1000 and 4000 cm⁻¹. The resulting absorbance peaks and their corresponding functional groups were identified.

2.4.4 Scanning (SEM) and transmission electron microscopy (TEM)

SEM was used to evaluate both the morphology of n-TiO₂ and fractured composite surfaces following the fracture toughness test (SEM, LEO (Zeiss) 1540XB). TEM was used to confirm the n-TiO₂ structure (TEM, Philips CM10). Samples were prepared for SEM by placing a small amount of fine n-TiO₂ powder on SEM stubs followed by coating with osmium in order to prevent charging of the sample and to reduce the nanotube damage from the electron beam during imaging. TEM sample preparation was done by adding a small amount of n-TiO₂ powder to an Eppendorf tube containing filtered deionized water. A drop of suspension was placed on a carbon grid and left to dry in air for 2 min then examined by TEM.

2.5 Preparation of composite specimens

Commercially available composite was used as control (Filtek™ Supreme Ultra Flowable Restorative, 3M ESPE). Composites were reinforced by incorporating different weight percentages (0.5, 1, 1.5, 2, 3, 5%) of n-TiO₂. Four different groups of composite were prepared. *Group A*: control flowable composite (no n-TiO₂ added); *Group B*: flowable composite reinforced with nonfunctionalized (NF) n-TiO₂; *Group C*: flowable composite reinforced with silane-functionalized n-TiO₂; *Group D*: flowable composite reinforced with MA-functionalized n-TiO₂. Composite disc specimens (5 mm diameter, 2 mm thickness) were prepared for ultrasonic, radiopacity, and cytotoxicity tests. Notchless triangular prisms (NTP, 6 x 6 x 6 x 12 mm) were prepared for fracture toughness tests. Composite discs were prepared by loading 1.2 g of each group of composite into 1 cc disposable syringes followed by light curing for 90 s (Dentacolor xs, KULZER). A microtome was used to cut the composite discs. NTP specimens were prepared by using a triangular prism Teflon mold. A small amount of composite (0.6 g) of each group was dispensed into the mold and a microscope glass slide was pressed against it to remove excess material. Afterwards, the composite was light cured and removed from the mold. Samples were soaked at 37°C in deionized water for 1 week prior to mechanical testing. The total number of composite specimens was 125 including 93 discs and 32 NTP specimens.

2.6 Assessment of flowability

The flowability testing method used in this study was according to ADA guidelines for evaluation of endodontic sealing materials [46]. To evaluate the flowability of the

reinforced composites, a simple test was done using the Gillmore needle apparatus (modified ADA approach). Different weight percentages of NF n-TiO₂ (0.5, 1, 1.5, 2, 3 and 5 %) were incorporated to a commercial flowable composite (Filtek™ Supreme Ultra Flowable Restorative, 3M ESPE). The quantity of 0.1 mL of control and each reinforced composite was dispensed between two thin microscopic cover glasses 50 x 50 mm (KODAK slide cover glass, Eastman Kodak Comp, NY, USA). These coverslips were then placed under a constant weight using 1 mm diameter needle weighing 454 g (Gillmore needle) for 30 s (i.e. compressive loading force of 4.45 N). As a result, the composite spreads forming an expanding disc (Fig. 2.1). The composite discs were subjected to light curing for 30 s and their diameters were measured and compared (the larger the diameter, the greater the flowability). The thickness of the cover glass that we used in this test was measured by a digital micrometer and found to be 0.62 mm. This thickness is substantially greater than the standard thickness of microscope cover glasses (0.12 mm) and hence was unlikely to deform during the test.

Fig. 2.1: Evaluation of flowability. Flowability was determined by measuring the diameters of composite discs between two microscope coverslips (a) Flowable composite dispensed on a coverglass on a flat platform, (b) covered with another coverslip, and (c) placed under a constant load for 30 s.

2.7 Mechanical testing of composites

2.7.1 Dynamic Young's moduli (E)

Dynamic Young's modulus was evaluated using a non-destructive ultrasound technique described elsewhere [62, 63]. The main idea of this technique is to analyze the time required for ultrasonic wave propagation. The instrument set up has the following components: a pulse generator, transmitting and receiving piezoelectric crystals (lithium niobate), amplifier, and oscilloscope (Fig. 2.2). During the test, a high-frequency pulse (10 MHz) is triggered by pulse function generator causing an ultrasonic mechanical vibration to be emitted by piezoelectric transducers. When the ultrasonic wave reaches the receiving piezoelectric element, it generates a similar frequency pulse at its electrodes which is recorded on the oscilloscope.

Fig. 2.2: Ultrasonic method setup. T is the time for ultrasonic wave to travel across the specimen and receiving crystal and d is the thickness of the specimen.

The specimen thickness (d) (mm) was measured using a digital wrench micrometer. The ultrasonic wave velocity was determined using the following equation: $C = d/t$ where C

is the velocity of sound (m/s), $t = T - \Delta t$ where T is the total travelling time of the ultrasonic wave across the sample and the receiving crystal (μs), and Δt is the travelling time of the ultrasonic wave through the receiving crystal (μs).

Poisson's ratio (ν) and Dynamic Young's modulus (E) were calculated using the following equations:

Poisson's ratio (ν) (dimensionless)

$$\nu = \frac{\left(\frac{1}{2}\right) \left(\frac{C_l}{C_s}\right)^2 - 1}{\left(\frac{C_l}{C_s}\right)^2 - 1}$$

where C_l = longitudinal wave velocity (m/s)

C_s = shear wave velocity (m/s)

Dynamic Young's modulus, E (GPa)

$$E = \frac{(1 + \nu)(1 - 2\nu)}{(1 - \nu)} (\rho C_l^2)$$

Where ρ = density of specimen (kg/m^3)

The density of each specimen (ρ) was determined using the Archimedes method.

2.7.2 Fracture toughness (K_{IC}):

Fracture toughness tests were performed on an Instron 3345 materials testing machine at a crosshead speed of 0.1 mm/min using NTP specimens [69]. The typical total test time was an average of 15 min/specimen. The NTP method uses small notchless triangular prism samples (6 x 6 x 6 x 12 mm). The K_{IC} value was obtained using the standard

formula [95], $K_{IC} = P_{max} \gamma_{min}^* / DW^{1/2}$, where P_{max} is the maximum load recorded at fracture (N); D is the specimen diameter (mm), W is the specimen length (mm), and γ_{min}^* is the dimensionless stress intensity factor coefficient minimum. Ruse *et al.* [69] reported these values for NTP specimens. $D = 12$ mm, $W = 10.5$ mm and $\gamma_{min}^* = 28$.

2.8 Radiopacity

Composite disc specimens of each group A, B, C and D were placed on size 4 occlusal film (KODAK INSIGHT Dental Film, F speed) together with 3 pieces of lead and an aluminum (Al) step wedge (10-step wedge, 0.5 mm/step). The film was exposed to an X-ray beam using a dental X-ray machine (Gendex GX 770) at 70 kV and 7 mA for 0.47 s. The distance between X-ray source and the film was 50 cm. Lead backing was placed under the film to minimize backscatter radiation. Following the exposure, the film was processed using an automatic processor. The radiograph was scanned (Epson V700) for radiopacity evaluation. A digital color meter (Apple computer, Power Mac G5) was used to measure the % RGB scale of the scanned radiographic images. The aluminum step wedge was used as an internal standard for measuring the equivalent radiopacity of different materials as compared to the thickness of the Al step wedge. A correction factor (CF) for background radiation was considered in the calculation of the corrected % RGB (% cRGB). $CF = white_{\%RGB} - lead_{\%RGB} = 100 - 88.5 = 11.5$. Thus, $\% cRGB = (\% RGB + CF) - background$. Linear regression analysis was conducted between the logarithm of (% cRGB) and the logarithm of aluminum thickness. Significant correlation was obtained ($r = 0.9723$) (Fig. 2.3). The linear regression equation is:

$$\text{Log } (\% \text{ cRGB}) = 0.4778 \log (\text{Al}) + 1.2212$$

$$\text{Log } (\text{Al}) = (\text{Log } (\% \text{ cRGB}) - 1.2212) / 0.4778$$

The radiopacity of each composite specimen in mm Al thickness was divided by the specimen thickness to obtain the value in terms of mm Al equivalent.

Fig. 2.3: Linear regression curve for Log % cRGB versus Log Al thickness.

2.9 *In vitro* cytotoxicity

All composite specimens were sterilized in low-temperature, radio-frequency glow discharge (RFGD) argon plasma using a PDC-32G plasma cleaner (Harrick Plasma, Ithaca, NY). The samples were placed on a sterile plate, the plate was placed inside the plasma cleaner. The samples were flushed twice with argon gas for 30 s followed by 3 min of rest to establish a full vacuum. Plasma cleaning was done by turning the plasma RF value to medium and opening the argon tank halfway for 4 min. Finally, the vacuum was stopped and samples were removed into sterile culture dishes.

Cytotoxicity assays were performed on NIH/3T3 mouse fibroblast cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (cell proliferation kit I MTT, Roche). Culture media was Dulbecco's Modified Eagle Medium (DMEM; Gibco, Catalog # 11995 Invitrogen) supplemented with 10% fetal bovine serum (FBS; Gibco, Catalog # 12483, Lot # 586696) and 1% antibiotic (Antibiotic-Antimycotic; Gibco, Catalog # 14040). A set of triplicate specimens for four different groups was used for plating different cell densities: 10×10^3 , 20×10^3 , 30×10^3 cells/cm² in a 96-well culture plate. The plates were incubated at 37°C in 5% CO₂ for 24 h. Subsequently, 10 µL of MTT labeling reagent was added and incubated for 4 h. Finally, 100 µL solubilizing solution was added and left overnight. The colorimetric absorbance was recorded using a microplate reader (Tecan, Durham, NC).

2.10 Statistical analysis

We used one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests at a significance level of $P < 0.05$ to analyze differences among groups.

Chapter 3

Results

3.1 Chemical characterization of titanium dioxide nanotubes

We first used powder X-ray diffraction (XRD) to investigate the crystalline phase of n-TiO₂. The test was repeated twice. The same diffraction peaks were observed. A prominent diffraction peak was observed at 25.1°, in addition to other peaks at 37.6°, 43.2°, 47.8° and 53.7° (Fig. 3.1). These diffraction peaks represent the anatase crystal phase of TiO₂ [96]. The peak at 31.9° represents sodium trititanate (Na₂Ti₃O₇) [97].

Fig. 3.1: XRD signal intensity (arbitrary units) versus diffraction angle 2θ (degrees). Letter A represents diffraction peaks corresponding to anatase crystal phase of TiO₂.

EDX was also performed after the alkaline hydrothermal reaction and calcination of the n-TiO₂ sample to determine the atomic proportions of Ti, O and Sr atoms. Four EDX images were captured at different sites of the same n-TiO₂ sample and the average weight percent of each element was calculated. It was found that Ti and O had the highest weight percent. These two elements correspond to TiO₂. Lower percentages of C and Na were found in the sample. A trace amount of Sr and Ni were also detected. Table 3.1 summarizes the weight % of each element.

Table 3.1: Elemental analysis of n-TiO₂ sample using EDX	
Element	Weight (%)
Ti	39.85 (2.43)
O	35.05 (1.90)
Sr	0.32 (0.15)
Na	3.65 (0.50)
C	20.95 (3.55)
Ni	0.15 (0.12)
Total	100 (0.004)

Data are mean $\pm$ (SD) of weight % of each element measured by EDX

Following n-TiO₂ functionalization, FTIR was conducted to investigate the chemical bonds formed between n-TiO₂ and the coupling agents. In case of silane functionalization, FTIR spectra exhibited a strong absorbance peak at 1725 cm⁻¹ and two more peaks at 2935 and 3477 cm⁻¹ (Fig. 3.2a). The absorbance peak at 1725 cm⁻¹ corresponds to the carbonyl group stretching mode in MPS suggesting its chemisorption on n-TiO₂ surface [44]. Both CH₃ and OH groups are corresponding to alkoxy group of MPS [98]. On the other hand, FTIR spectra of MA functionalized n-TiO₂ showed absorbance bands at 1381 and 1633 cm⁻¹ in Fig. 3.2 (b). These peaks are due to the presence of CH₃ groups and the vinyl (C=C) bond of methacrylic acid coordinated to the n-TiO₂ respectively [25]. The absorbance peak at 2355 cm⁻¹ corresponds to the carboxylic group [99], while the broad peak at 3412 cm⁻¹ is due to hydrogen bonded OH stretch [100]. The FTIR was conducted twice using two different samples for both silane

and MA functionalized n-TiO₂. The results of both samples were the same.

Fig. 3.2: FTIR absorbance peaks and their corresponding functional groups for (a) n-TiO₂ functionalized with silane, (b) n-TiO₂ functionalized with MA.

3.2 Morphological characterization of titanium dioxide nanotubes

The morphology of n-TiO₂ was examined by SEM and TEM after hydrothermal reaction and calcination. SEM images revealed the presence of elongated tubular structures (Fig. 3.3a). TEM images confirmed the multi-walled hollow tubular structure of n-TiO₂. Inner and outer diameters were measured at three different samples ($n=3$). The average inner and outer diameters were 12.4 ($\pm$ 0.59) nm and 25.5 ($\pm$ 1.46) nm respectively (Fig. 3.3b).

Fig. 3.3: (a) SEM, (b) TEM of n-TiO₂ following alkaline hydrothermal reaction and calcination.

3.3 Flowability of reinforced and control composites

Flowability was evaluated by comparing the composite disc diameters after they have been sandwiched between two glass coverslips, subjected to constant weight (454 g) for 30 s and light cured for 30 s. Measuring the composite discs diameters was repeated 10 times at different points on the same sample (Figs. 3.4 and 3.5). Composite flowability decreased slightly with increasing amounts of NF n-TiO₂ fillers.

Fig. 3.4: Changes in composite discs diameter with different amounts of NF n-TiO₂ fillers. Disc diameters and flowability decreased slightly with increasing NF n-TiO₂ content.

Fig. 3.5: Flowability of control and reinforced composites was evaluated by measuring composite disc diameter. Data are disc diameters (measured 10 times at different points on the same sample). Line was fit by linear regression. *P* value indicates a significant linear correlation.

3.4 Mechanical characterization of reinforced and control composites

3.4.1 Dynamic Young's moduli (E)

The dynamic Young's modulus (E) of control and reinforced composites was determined using a non-destructive ultrasonic pulse method ($n = 3$). Three specimens of each filler weight % (0.5 to 5%) for each group (A, B, C, and D) were used. Composites reinforced with 3% MA functionalized n-TiO₂ exhibited the greatest elastic moduli of 16.8 GPa. E of composites reinforced with n-TiO₂ functionalized with 3% silane was 16.1 GPa. Both of them were significantly higher than that of control composite (10.6 GPa) at $P < 0.05$. However, composites reinforced with 3% NF n-TiO₂ showed lower E values (15.5 GPa) than those of the functionalized ones (Fig. 3.6). These results established that the largest Young's moduli were obtained when composite was reinforced with 3% n-TiO₂. Moreover, these data confirmed the successful effect of functionalization.

Fig. 3.6: Dynamic Young's moduli of reinforced composites as compared to control (0% filler content). (a) NF n-TiO₂, (b) n-TiO₂ functionalized with silane, and (c) n-TiO₂ functionalized with MA. Data are means $\pm$ SEM ($n = 3$) for each filler weight % in each group of composite. The same lower case letters indicate values that are not significantly different from each other ($P > 0.05$). **** and *** show significant differences at ($P < 0.0001$) and ($P < 0.001$) respectively. * shows significant differences at ($P < 0.05$).

3.4.2 Fracture toughness (K_{IC})

Fracture toughness (K_{IC}) and the maximum load applied to each specimen at fracture were determined using an Instron testing machine by applying tension ($n = 8$). NTP specimens were used for this test. Eight specimens of control composite and 3% n-TiO₂ reinforced composite (3% NF, 3% silane, and 3% MA) were tested. Composite reinforced with 3% MA achieved the highest value of K_{IC} , being statistically different from control and NF groups ($P < 0.05$). The maximum load value is the maximum force that the material can withstand prior to fracture due to crack propagation. Table 3.2 shows the measured maximum load values and K_{IC} values of control and reinforced composites.

Table 3.2: Fracture toughness (K_{IC}) of control and reinforced flowable composites				
	Control composite	Reinforced composite		
	0 %	3% NF	3% silane	3% MA
Maximum load (N)	30 (1.3)	41 (2.4)	46 (2.3)	52 (3.5)
K_{IC} (MPa.m^{0.5})	0.87 (0.03) ^a	0.93 (0.05) ^a	1.05 (0.05) ^{ab}	1.18 (0.08) ^b

Data are means (SEM). The same superscript letters indicate values that are not significantly different from each other ($P > 0.05$).

SEM was used to evaluate fracture surfaces of composites after fracture toughness testing. Spherical nanoparticles were observed in control composite (Fig. 3.7a). Composites reinforced with NF n-TiO₂ had nanotubes distributed throughout the composite matrix (Fig. 3.7b). However, composites reinforced with silane and MA functionalized n-TiO₂ showed evidence of nanotubes intimately embedded in the

composite matrix (Fig. 3.7c and d, respectively).

Fig. 3.7: SEM images of composite fracture surfaces. (a) control, (b) reinforced with NF n-TiO₂, (c) reinforced with n-TiO₂ + silane, and (d) reinforced with n-TiO₂ + MA.

3.5 Radiopacity of reinforced and control composites

Radiopacity of composites was measured in terms of mm aluminum equivalent ($n = 3$).

Three specimens of control and each group of reinforced composite (at 3%) were used (Fig 3.8).

Fig. 3.8: Radiopacity of flowable composite specimens, leads and Al step wedge. (A) Control composite, (B) composite reinforced with NF n-TiO₂, (C) composite reinforced with n-TiO₂ + silane and (D) composite reinforced with n-TiO₂ + MA.

We evaluated the radiopacity by using the digital color meter method in which the % RGB was used to calculate the radiopacity (mm Al thickness). To calculate the radiopacity in terms of the Al equivalent, radiopacity values were divided by each specimen thickness. Fig. 3.9 displays the radiopacity of the control and reinforced flowable composites. It can be seen that the control composite exhibited the highest radiopacity value of 2.62 mm Al. NF and silane groups are comparable to control (2.19 mm Al). However, the radiopacity of MA group (1.64 mm Al) was significantly lower than that of control ($P < 0.05$).

Fig. 3.9: Radiopacity of flowable composites (mm Al). Data are means $\pm$ SEM ($n = 3$). The same lower case letters indicate values that are not significantly different from each other ($P > 0.05$).

3.6 Biocompatibility of reinforced and control composites

NIH/3T3 fibroblasts were plated on composite specimens for each group. Three independent experiments were done at three different points of time, each with a triplicate set of specimens for control, NF, silane and MA groups ($n = 3$). The fibroblasts were plated in three different cell densities (10×10^3 , 20×10^3 and 30×10^3 cells/cm²) and cultured for 24 h. Fibroblast viability was then assessed using the MTT assay. In this assay, cell viability is directly proportional to colorimetric absorbance of the MTT dye. The results showed comparable numbers of cells on control and reinforced composites (no significant differences, $P > 0.05$), indicating that these composites are biocompatible *in vitro* (Fig. 3.10).

Fig. 3.10: MTT cytotoxicity test. NIH/3T3 fibroblasts were plated at different densities (10, 20, 30 x 10³ cells/cm²) on control, NF, silane- and MA-reinforced composites and incubated for 24 h. The MTT dye absorption is directly proportional to the viability of the cells. No significant differences were found in cell viability among each cell plating density. Three independent experiments were done at three different points of time, each with a triplicate set of specimens for control, NF, silane and MA groups ($n = 3$).

Chapter 4

Discussion

4.1 Nanocomposite fillers

Nanocomposite materials continue to be investigated for their potential roles in restorative dentistry – for their qualities associated with handling properties, mechanics, biocompatibility and esthetics. They are an interesting topic for research in the field of dental materials. Many studies are being done in order to understand their properties and how they differ from other composites. Different nanofillers have been introduced to dental composites.

Hua *et al.* [52] studied the effect of incorporating TiO₂ nanoparticles in dental composites. They concluded that Young's modulus showed much more rapid increase by adding nanoparticles than by adding glass fibers. Increasing Young's modulus by 30% required 3% volume fraction of nanoparticles, whereas 6% of glass fibers were needed to achieve the same increase in stiffness. This is likely due to the large specific surface area of nanoparticles, which could greatly facilitate load transfer from resin matrix to nanoparticles resulting in better mechanical properties of nanocomposites than microcomposites.

Composite reinforcement with nanofibers was studied by Tian *et al.* [101] in which nanofibers (containing fibrillar silicate crystals) were added to Bis-GMA/TEGDMA composite. The average diameter of the nanofibers was 250 nm. Their results revealed that adding small mass fractions of nanofibers (1-2%) improved the mechanical properties of the composite including elastic modulus, fracture toughness and flexural strength; whereas, larger fractions of 4-8% resulted in inferior mechanical properties. Our results are in line with these. We found that by incorporating small weight fractions of nanotubes (up to 3%) to a flowable dental composite improved its dynamic

Young's modulus (E). However, at 5 wt% n-TiO₂, E started to decrease, likely as a result of agglomeration.

Another study tried to improve the mechanical properties of dental composites by reinforcing them with TiO₂ nanoparticles (< 20 nm) functionalized with allyltriethoxysilane (ATES) [40]. The mechanical properties of these composites were compared to those of composites reinforced with non-functionalized (NF) TiO₂ nanoparticles and to composites with no TiO₂ nanoparticles. It was reported that TiO₂ nanoparticles functionalized with ATES improved the composite flexure strength. However, the NF nanoparticles had a limited effect on the mechanical properties of dental composites because of their agglomeration.

4.2 Synthesis of n-TiO₂ using the alkaline hydrothermal reaction

Formation of n-TiO₂ during alkaline hydrothermal reaction takes place due to formation of nanosheets with high curling tendency [102]. As described by Kasuga *et al.* [30] these sheets are formed due to the reaction between the acid and Ti–O–Na with a decrease in Ti–O–Ti bond length. They suggested that the residual electrostatic repulsion of Ti–O–Na bonds may have an influence in connecting Ti–O–Ti sheets followed by their closure and nanotubes formation (Fig. 4.1).

Many studies were conducted to understand the effect of hydrothermal temperature. Yuan and Su [103] studied nanostructures at different hydrothermal temperatures. They concluded that hydrothermal reaction temperature of 100–160°C results in nanotube formation, whereas higher temperature of 180–250°C leads to formation of nanoribbons. Ribbon-like structures were obtained from hydrothermal

reactions at 200°C [104]. Our results are in agreement with these results. In our work, the hydrothermal reaction was carried out at 160°C and resulted in nanotube formation.

As reported earlier by Kasuga *et al.* [30], SEM images showed needle-shaped structures of HCl-treated samples following alkaline hydrothermal method. They also reported that the specific surface area of n-TiO₂ greatly increased after washing with distilled water and HCl. In agreement with that, Liu *et al.* [105] found that the surface area of n-TiO₂ was 78 m²/g following the alkaline hydrothermal reaction. However, the surface area was increased to 330 m²/g after washing with HCl. This confirms the fact that this reaction has a remarkable effect on the surface area of nanotubes. Moreover, Kasuga and coworkers [30] reported TEM images that indicated the presence of wide lattice stripes at both ends of the needle-shaped structures. They reported nanotubes with inner and outer diameters of 5 nm and 8 nm, respectively. In our study, the nanotubes had greater inner and outer diameters, 12 nm and 25 nm, respectively. This may have been due to the higher temperature (160°C) that we applied during the alkaline hydrothermal reaction.

Fig. 4.1: TiO₂ Nanotube formation scheme.

4.3 Filler functionalization

4.3.1 Purpose of functionalization

Functionalization of fillers is important in composites to provide a link between the polymer matrix and fillers [4]. In the case of nanofillers, functionalization is crucial to avoid nanofiller agglomeration because of their small size. Silane has been used frequently as a coupling agent in dental composites. In the studies reported in this thesis, we used two different methods for functionalizing n-TiO₂. We used a dual-silanization method and methacrylic acid method. The latter has been reported to be a functionalization agent for n-TiO₂ in bone cements [25].

4.3.2 Functionalization with silane

Wilson *et al.* [38] described the dual-silanization method using MPTS and OTMS. This method resulted in improved composite handling properties, lower polymerization shrinkage and improved durability in the oral environment. These effects are more prominent in the case of nanocomposites than in conventional composites that have higher filler content. This is likely due to the fact that nanofillers have a much larger surface area per unit mass [34].

Another study was carried out by Karebela *et al.* [106] to compare the absorption of water or ethanol/water solution in dental nanocomposites containing silica nanoparticles silanized with different silanes including urethane dimethacrylate silane (UDMS), MPTS, and OTMS. Composite absorption of water or ethanol/water solution was found to be influenced by the structure of silane. Composites with UDMS, which has a hydrophilic urethane group, showed the highest amount of water absorption. On the other hand, composite with OTMS that does not have a methacrylate moiety (i.e, can not

react with dimethacrylate monomers) was found to have highest solubility, while the MPTS composite was found to absorb the lowest amount. They concluded that the structure of silane affects the solubility of composites.

In other studies, nanosilica particles functionalized with glycidoxypropyltrimethoxy-silane (GPS) were found to be well-dispersed in resin matrix without aggregation. In contrast, SiO_2 nanoparticles without GPS aggregated to form macrosized clusters [8]. In our studies, FTIR of n-TiO₂ functionalized with silane showed a peak at 1725 cm^{-1} due to the C=O vibration band in addition to other peaks corresponding to alkoxy group. The C=O vibration band is associated with carbonyl group from MPS. The other peaks at 2934 and 3477 cm^{-1} are due to CH_3 and OH stretches corresponding to alkoxy group of MPS, which is able to bind with the hydroxyl group on the surface of n-TiO₂. Fig. 4.2 shows a schematic for binding between MPS and n-TiO₂. $\text{R-Si}(\text{OCH}_3)_3$ represents a general silane molecule where R is an organic functional group (such as methacrylate) that forms a covalent bond with the organic resin matrix [107, 108].

Fig. 4.2: Deposition of MPS on n-TiO₂. This reaction takes place via hydrolysis followed by polycondensation.

4.3.3 Functionalization with methacrylic acid (MA)

MA is a bifunctional agent, which is able to form a chemical link between n-TiO₂ and the polymer matrix through a Ti-carboxylic coordination bond [25]. In our studies, FTIR for n-TiO₂ functionalized with MA showed an absorbance peak at 1633 cm⁻¹ due to the vinyl bond of methacrylic acid. Another peak at 2355 cm⁻¹ corresponds to carboxylic groups of

MA coordinated with Ti atoms. The peaks at 1381 and 3412 cm^{-1} are due to CH and OH stretches respectively. They also act as functional sites for further copolymerization, Fig. 4.3 [109]. The presence of these peaks that represent the functional groups and bond formation between n-TiO₂ and MA provide evidence of chemical bond formation between these functionalized fillers and the resin matrix.

Fig. 4.3: Schematic of MA functionalization of TiO₂. Adapted with permission (Appendix A) from the Langmuir. 2007;23:3988-95. [109]

4.4 Chemical characterization of n-TiO₂

Our XRD results confirmed that titania nanotubes prepared by alkaline hydrothermal technique and calcined at 400°C exhibit the anatase phase of TiO₂ [110, 111]. Sodium trititanate (Na₂Ti₃O₇) was detected in TiO₂ samples. This could be a transition phase between hydrothermal treatment and HCl washing. Similar results were reported in previous studies. Jiang *et al.* [110] found anatase diffraction peaks corresponding to TiO₂ calcined at 400°C . Yu and coworkers [112] reported anatase phase of TiO₂ for samples calcined at $300 - 600^{\circ}\text{C}$. However, at the higher temperature of 700°C , anatase and rutile phases were found. At the higher calcination temperature of 800°C , only the rutile phase was seen [113].

EDX was done for n-TiO₂ following alkaline hydrothermal reaction and calcination. It is clearly seen that Ti and O have the highest weight per cent. These two elements correspond to TiO₂. A small percentage of Na was found in the sample indicating that a small portion of Na ions (from NaOH used during the alkali treatment step of n-TiO₂ synthesis) remained after washing. A trace amount of Sr was also detected. However, C and Ni likely came from the coating and grids that were used to mount the samples.

4.5 Flowability

Flowability testing method used in this study was described earlier by Bayne *et al.* [19] to compare different dental flowable composites. They concluded that the ideal flowable composite should not flow because of its own weight; instead, it should require a force to start to flow. Lee *et al.* [45] studied the rheological properties of flowable and other composites. They concluded that composite viscosity increases with increasing amounts of fillers, but no direct linear relation was found. Instead, there is a weak relationship between filler volume % and composite viscosity. In accordance with these results, Beun and coauthors [114] confirmed no correlation between the viscosity of flowable dental composites and the filler weight fraction. They suggested that adding nanoparticles could have a positive effect on flowable composites in terms of mechanical properties and better flow. This is applicable to our titania nanotubes. Previous studies reported that it is possible to increase the nanofiller content due to their small particle sizes. This will result in significant reduction in polymerization shrinkage as well as improvement in the physical properties of the nanocomposites [8].

4.6 Mechanical properties of composites

4.6.1 Dynamic Young's modulus (E)

Dynamic Young's modulus plays an important role in the stresses generated during polymerization shrinkage of resin composite. This phenomenon is critical in terms of the success or failure of adhesive restorations because shrinkage results in marginal gap formation, discoloration, recurrent caries and post-operative sensitivity [115].

To measure E, we used the ultrasonic pulse method. This technique has the advantage of being non-destructive, permits the use of small sample size, and it can be used to measure the material's rigidity. E is an important parameter to assess the effect of nanotube functionalization, as it gives an idea about the adhesion between functionalized n-TiO₂ and composite matrix [62]. Basically, the method works by applying an ultrasonic pulse through the composite sample in both longitudinal and transverse waves and then measuring their velocities [116]. We found that composite reinforced with 3% MA functionalized nanotubes has the highest Young's modulus (16.8 GPa), which is significantly higher than that of control composite (10.6 GPa).

The E value of control composite measured in our work is in the range of other commercial flowable composites studied by Sabbagh *et al.* [56]. They measured the dynamic elastic moduli for composite specimens after incubation in distilled water at room temperature for 7 d. They reported E values for flowable composites ranging between 4.6 - 10.9 GPa. E for packable composites were found to be in the range of 8.3 – 20.3 GPa. However, E of amalgam was reported to be 30 GPa [2]. According to Jones *et al.* [62], the higher magnitude of Young's moduli reflects the stronger adhesion between n-TiO₂ and composite matrix with a higher extent of functionalization.

Understanding the mechanical properties of human teeth is important for the development of restorations with characteristics closer to those of natural teeth [56, 117]. When replacing a tissue, the ideal material for replacement should have a modulus of elasticity similar to that of replaced tissue. In terms of dental restorations, they should have a modulus of elasticity similar to that of dentin (the most abundant mineralized tissue in teeth). However, an optimal restoration should mimic both dentin and enamel in structural, mechanical and physical properties. This is impossible to achieve unless different restorative materials are used in combination [115]. Less marginal breakdown is expected with smaller differences in the elasticity [118, 119].

Many studies have reported E values for enamel and dentin. Xu *et al.* [117] measured elastic modulus of human enamel and dentin. They found that Young's modulus of enamel ranges between 80-94 GPa depending on tooth orientation. However, Young's modulus of dentin found to be 19 GPa. Craig and Peyton [49] reported 18 GPa elastic modulus value for dentin in human. Elastic modulus values of 15 GPa for dentin and 40-80 GPa for enamel were reported by Rees and Jacobsen [120]. McCabe [2] reported E values of 50 GPa and 12 GPa for enamel and dentin respectively.

Braga *et al.* [121] studied the effect of composite modulus of elasticity on stress distribution at the tooth-restoration interface. They concluded that stress concentration decreased with composites that have a modulus value of 12 GPa due to less modulus mismatch between dentin and composite. It is clearly seen that the E values of our reinforced composite samples are comparable to that of human dentin. Furthermore, applying a flowable composite lining underneath the main filling could be effective in reducing shrinkage stress [55]. The lower elastic moduli and the higher flowability of

composites might allow stress concentration relaxation and possible reduction of marginal breakdown and debonding [56]. Our reinforced flowable composite could be ideal for this purpose as the reinforcing nanotubes may greatly reduce its polymerization shrinkage.

4.6.2 Fracture toughness (K_{IC})

Fracture toughness is one of the most important mechanical properties for dental materials. It is defined as the energy absorbed by the material prior to fracture due to crack propagation. In case of poor adhesion between fillers and matrix, debonding will occur during tensile loading resulting in the formation of microvoids within the matrix with subsequent crack propagation [25]. In our study, this was investigated by SEM of composite fracture surfaces. The covalent bond formed between functionalized n-TiO₂ and the composite matrix appeared to influence their adhesion and embedding in the composite matrix.

Schultz *et al.* [122] compared K_{IC} of a few commercial highly filled composites with their corresponding flowable ones. They reported a highly filled (Nanopaq) composite to have the highest K_{IC} value of 3.64 MPa.m^{0.5}. On other hand, Dyract flowable composite showed the lowest K_{IC} value of 0.82 MPa.m^{0.5}. Generally, all the tested flowable composites showed significantly lower K_{IC} values as compared to their corresponding highly filled ones. In case of the n-TiO₂ we used in this study, K_{IC} were 0.93, 1.05 and 1.18 MPa.m^{0.5} for composites reinforced with NF n-TiO₂, composites reinforced with n-TiO₂+silane and composites reinforced with n-TiO₂+MA, respectively. Soderholm [64] reviewed the K_{IC} values for enamel, dentin and different

dental materials. He reported the K_{IC} values ranging from 0.65 – 2.5 MPa.m^{0.5} for enamel and 1.0 - 4.0 MPa.m^{0.5} for dentin. K_{IC} values of dental amalgam were found to be in the range of 1.4 to 2.4 MPa.m^{0.5}, while K_{IC} value for composites ranged between 0.7 and 1.9 MPa.m^{0.5}. In another study, K_{IC} values for enamel were between 0.6 and 1.8 MPa.m^{0.5} and 3.1 MPa.m^{0.5} for dentin [51].

Table 4.1 summarizes the E and K_{IC} values for enamel, dentin and different dental materials. Previous studies found that reinforcing fillers could distribute the force propagation into several components causing crack curving or dissipation between particles. This will create conditions unfavourable for growth of the crack. However, the relationship between the filler content and K_{IC} seems to be complicated [51].

Table 4.1: Summary for E and K_{IC} values for enamel, dentin and different dental materials		
	E (GPa)	K_{IC} (MPa.m^{0.5})
Enamel	40 - 94	0.65 – 2.5
Dentin	12 - 19	1.0 – 4.0
Amalgam	30	1.4 – 2.4
Packable composites	8.3 – 20.3	0.7 – 1.9
Flowable composites	4.6 – 10.9	0.82
Our n-TiO ₂ flowable composite	15.5 – 16.8	0.93 – 1.2

4.7 Radiopacity

The radiopacity of a restorative material is an important parameter for accurate diagnosis and treatment planning. Evaluating long term success or failure of the restoration is highly dependent on radiographs. Composite restorations radiopacity has an important role in detecting recurrent caries and distinguishing the restorations from the natural tooth structures [78]. The radiopacity of dental composites according to the ISO standard 4049 should be equal to dentin, and equal or greater than 1 mm Al equivalent [75].

Previous studies suggested that the composite first increment should have a sufficient radiopacity to clearly evaluate the tooth-restoration interface. This is important when flowable composite is used as an intermediate layer between the restoration and the tooth [123]. Other studies recommended that the composite radiopacity should be equal to or greater than that of the enamel [17, 76, 78, 123]. However, materials with much higher radiopacity than enamel (such as amalgam) may lead to under or over estimation of secondary caries and marginal defects. Thus, materials with slightly higher radiopacity than enamel are preferred [74, 124]. The amalgam is too radiopaque as compared to the tooth. This is associated with the risk of undiagnosed caries and adjacent defects, which are hidden in the shadow of amalgam [125].

Matsumura *et al.* [125] reported the radiopacity values of different dental materials as examples; enamel (1.77 – 2 mm Al), dentin (0.98 – 1.01 mm Al) and amalgam (9.74 – 11.47 mm Al). Flowable composites were found to have lower radiopacity as compared to hybrid composite due to their lower filler content [126]. Sabbagh and others reported linear correlation between the filler weight percent and radiopacity [127]. The atomic number of the elements is the most important factor

affecting the radiopacity of dental materials [128]. Radiopacity of dental composites can be increased by incorporating a higher percentage of fillers with high atomic numbers such as barium, strontium and ytterbium [127].

Furtos and co-authors [129] evaluated the radiopacity of 17 different flowable composites. The radiopacity values ranged between 1.14 and 3.57 mm Al. Dukic and co-workers [130] reported the radiopacity for 32 dental composites (including both microfilled and nanohybrid). The radiopacity ranged from 0.61 to 4.78 mm Al. In our study, the radiopacity of n-TiO₂ reinforced flowable composites ranged between 1.64 and 2.19 mm Al. The control composite showed a higher value of radiopacity (2.62 mm Al). However, all of these composites (control, NF n-TiO₂-reinforced composites, composites reinforced with n-TiO₂ + silane and composites reinforced with n-TiO₂ + MA) met the ISO standard for dental materials radiopacity. All of them had radiopacity greater than dentin. Table 4.2 summarizes the radiopacity values of enamel, dentin and different dental materials.

Table 4.2: Radiopacity of enamel, dentin and restorative materials	
Tissue/ Material	Radiopacity (mm Al)
Enamel	1.77 – 2.00
Dentin	0.98 – 1.01
Amalgam	9.74 – 11.47
Packable composites	0.61 - 4.78
Flowable composites	1.14 – 3.57
Our n-TiO ₂ flowable composites	1.64 – 2.19

4.8 Biocompatibility

All dental materials, including resin composites, are required to be tested *in vitro* and *in vivo* to evaluate the biocompatibility. In our study, we evaluated possible cytotoxicity using the MTT assay to evaluate fibroblast viability. Fibroblasts are an approved cell type for cytotoxicity testing by ISO. In addition, they are the most dominant cell type in the pulp that can be affected by the leaching components from composite restorations [83]. The MTT cytotoxicity assay is a rapid, sensitive, colorimetric test [131]. This assay utilizes mitochondrial dehydrogenase activity that results in cleavage of a yellow tetrazolium salt (MTT) to form purple formazan crystals [132]. MTT dye reduction appears to take place only in viable cells; in contrast, nonviable cells cannot reduce the dye [133].

Our MTT assay results showed no significant difference in the viability of cells cultured on pure and reinforced composites. These data indicate that our reinforced composites have excellent biocompatibility. Assessment of biocompatibility using dye uptake methods provides an idea only about cell death caused by biomaterials. However, biomaterials may show different mechanisms of cytotoxicity. Therefore, biocompatibility should be assessed using a number of quantitative measures. Ciapetti *et al.* [134] recommended a model proposed in xenobiotic research. It utilizes the LDH assay for evaluation of membrane integrity, ^3H -thymidine uptake to quantify DNA synthesis, neutral red uptake to evaluate the efficiency of lysosomes, the Lowry method for protein assessment, and the MTT assay to test mitochondrial activity. Using such a system to study cell-biomaterial interactions will be valuable in future studies.

Chapter 5

Conclusions and Future Directions

5.1 Summary and conclusions

A flowable dental composite was successfully reinforced with n-TiO₂. Incorporation of small weight percentages of this nanofiller exhibited a marked improvement in the mechanical properties. The flowability of the reinforced composites was slightly reduced, but was acceptable for clinical applications. Our research findings are summarized as follows:

- n-TiO₂ powder was successfully synthesized by alkaline hydrothermal reaction at 160°C to act as a reinforcing phase for a flowable dental composite.
- Three different groups of n-TiO₂ were prepared – i) nonfunctionalized (NF) n-TiO₂, ii) n-TiO₂ functionalized with silane, and iii) n-TiO₂ functionalized with methacrylic acid (MA) – to evaluate the reinforcing effect of each group.
- Chemical characterization of n-TiO₂ was conducted using XRD, EDX and FTIR. XRD showed anatase diffraction peaks of n-TiO₂. EDX showed Ti and O had the highest weight %. FTIR revealed that both silane and MA were successful coupling agents for n-TiO₂ fillers. FTIR absorption spectra showed the presence of functional groups corresponding to binding between n-TiO₂ and the functionalizing agent. Bond formation between silane and n-TiO₂ was primarily through alkoxy group. However, carboxylic group resulted in bond formation between MA and n-TiO₂.
- SEM and TEM images of n-TiO₂ exhibited the tubular morphology with no evidence of agglomeration. SEM of composite surfaces following fracture toughness test shows gaps at the interfaces between NF n-TiO₂ and the resin matrix. On the other hand, functionalized n-TiO₂ (both silane and MA) were embedded within the

composite resin matrix. However, SEM images of fracture surfaces of control composite showed only spherical nanoparticles embedded into the resin matrix.

- n-TiO₂ were successfully incorporated into a flowable composite. Nanotubes were incorporated in small weight fractions as they have a high surface area.
- Flowability evaluation of composites showed a minimum decrease in composite fluidity with increasing n-TiO₂ content.
- Mechanical properties of the reinforced composites were significantly higher than those of control composite. Flowable composites reinforced with MA-functionalized n-TiO₂ exhibited the highest values of elastic moduli. Both silane- and MA-functionalized n-TiO₂ had E values significantly greater than that of control composite. E values of flowable composites reinforced with n-TiO₂ are comparable to that of human dentin. Moreover, these reinforced flowable composites showed higher resistance to crack propagation. Composite reinforced with 3% MA achieved the highest value of K_{IC}, significantly greater than the control and NF groups.
- Although the radiopacity of the MA-reinforced composite was significantly less than that of the control composite, all composites met the ISO standard for radiopacity of dental restorative materials.
- *In vitro* cytotoxicity testing using MTT showed excellent biocompatibility of n-TiO₂-reinforced composites. The viability of cells grown on the reinforced composites was comparable to that of cells grown on the control composite.
- A reduction in polymerization shrinkage of flowable composites is expected with the incorporation of n-TiO₂ fillers, due to their high surface area to volume ratio.

- Improved mechanical properties, acceptable handling characteristics and excellent biocompatibility of flowable composites reinforced with n-TiO₂ make them promising materials for future applications in restorative dentistry.

5.2 Future directions

Although n-TiO₂ reinforced flowable dental composites could result in successful restorations, it is recommended to conduct the following tests to predict their long-term performance.

- Wear resistance and surface polish of n-TiO₂ reinforced composites should be evaluated.
- As the polymerization shrinkage is expected to decrease in composites reinforced with n-TiO₂, these restorations could be tested in extracted teeth. Histologic sections can be done afterwards to evaluate marginal leakage at the tooth-restoration interface.
- Our reinforced composites showed excellent biocompatibility *in vitro*. Future studies should examine their biocompatibility *in vivo*. Animal model for dental materials biocompatibility testing includes rats, rabbits, dogs and non-human primates [79, 135]. Favorable outcomes in animal models could then lead to an application to Health Canada to undertake clinical trials in humans.

It is also recommended to reinforce other dental resins with TiO₂ nanofillers in order to improve their physical and mechanical properties as the following:

- Changing the temperature and pH of the hydrothermal reactions could produce different TiO₂ nanofillers. We suggest incorporating TiO₂ nanoribbons (can be

fabricated at 180–250°C hydrothermal temperature) and ribbon-like structures (can be fabricated at 200°C under hydrothermal conditions) to flowable dental composite and compare their effects to that of the n-TiO₂.

- Incorporation of a small weight fraction of n-TiO₂ fillers to dentin bonding agents to render them radiopaque. The dentin bonding agents are known to be radiolucent or have lower radiopacity than dentin. This makes the radiographic examination and diagnosis challenging in terms of recurrent caries detection. Addition of n-TiO₂ doped with Sr to dentin bonding agents could facilitate their detection in radiographs.

Chapter 6

References

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ACS Publications
High quality. High impact.

Title: Structure Analysis of Nanowires and Nanobelts by Transmission Electron Microscopy
Author: Yong Ding and and Zhong Lin Wang*
Publication: The Journal of Physical Chemistry B
Publisher: American Chemical Society
Date: Aug 1, 2004
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ACS Publications **Title:**
High quality. High impact.

Synthesis of TiO₂-PMMA
Nanocomposite: Using
Methacrylic Acid as a Coupling
Agent

Author: S. M. Khaled,[†] Ruohong Sui,[†]
Paul A. Charpentier,^{*,†} and
Amin S. Rizkalla^{†,‡}

Publication: Langmuir

Publisher: American Chemical Society

Date: Mar 1, 2007

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Appendix B

Composition of Filtek™ Supreme Ultra Flowable Restorative, 3M

ESPE as Supplied by Manufacturer

MATERIAL SAFETY DATA SHEET 6032/ 6033 3M™ ESPE™ FILTEK™ SUPREME ULTRA FLOWABLE RESTORATIVE
03/14/13

Material Safety Data Sheet

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SECTION 1: PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: 6032/ 6033 3M™ ESPE™ FILTEK™ SUPREME ULTRA FLOWABLE
RESTORATIVE

MANUFACTURER: 3M

DIVISION: 3M ESPE Dental Products

ADDRESS: 3M Center, St. Paul, MN 55144-1000

EMERGENCY PHONE: 1-800-364-3577 or (651) 737-6501 (24 hours)

Issue Date: 03/14/13

Supersedes Date: 05/04/10

Document Group: 27-9483-2

Product Use:

Intended Use: Dental product
Limitations on Use: For use only by dental professionals
Specific Use: Composite restorative material

SECTION 2: INGREDIENTS

<u>Ingredient</u>	<u>C.A.S. No.</u>	<u>% by Wt</u>
SILANE TREATED CERAMIC	444758-98-9	50 - 60
SUBSTITUTED DIMETHACRYLATE	27689-12-9	15 - 25
BISPHENOL A DIGLYCIDYL ETHER DIMETHACRYLATE (BISGMA)	1565-94-2	5 - 10
SILANE TREATED SILICA	248596-91-0	5 - 10
TRIETHYLENE GLYCOL DIMETHACRYLATE (TEGDMA)	109-16-0	5 - 10
Ytterbium fluoride (YbF ₃)	13760-80-0	< 5
FUNCTIONALIZED DIMETHACRYLATE POLYMER	None	< 5
TITANIUM DIOXIDE	13463-67-7	< 0.5
ETHYL 4-DIMETHYL AMINO BENZOATE (EDMAB)	10287-53-3	< 0.5
BENZOTRIAZOL	96478-09-0	< 0.5
DIPHENYLIODONIUM HEXAFLUOROPHOSPHATE	58109-40-3	< 0.5

Curriculum Vitae

Name: Manal Dafar

Post-secondary Education and Degrees:

M.Sc. candidate, Medical Biophysics Graduate Program
Schulich School of Medicine & Dentistry
Western University
London, Ontario, Canada
2012 – 2014

Bachelor Degree in Dental Medicine and Surgery (B.D.S)
King Abdulaziz University
Jeddah, Saudi Arabia
2002 - 2008

Honours and Awards:

Scholarship for graduate and clinical studies
Ministry of Health, Saudi Arabia
2012 - 2014

Certificate of achieving second honour degree
School of Dentistry, King Abdulaziz University
Jeddah, Saudi Arabia
2008

Related Work Experience:

Dental resident
King Fahad General Hospital
Jeddah, Saudi Arabia
2010 – 2011

Dental intern
King Abdulaziz University Hospital
Jeddah, Saudi Arabia
2008 – 2009

Posters and presentations:

March 2014: Mechanical properties and cytotoxicity of flowable dental composites reinforced with TiO₂ nanotubes. London Health Research Day, London ON, Canada.

June 2013: Flowable dental composites; indications and limitations. UWO, London ON, Canada

March 2012: Dental implant biomaterials, UWO, London ON, Canada

March 2009: Hyperbaric oxygen therapy in dentistry, Department of Maxillofacial Surgery, King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia

Nov 2007: Fracture resistance of all-ceramic restorations, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia