

**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE  
AND PHARMACY TIMIȘOARA  
FACULTY OF DENTAL MEDICINE  
DEPARTMENT I**

**TOFAN SERGIU ALEXANDRU**



# **PHD THESIS**

**STUDIES REGARDING THE ROLE OF NANOCOMPOSITES  
AND POLYMERIC BIOMATERIALS IN DENTAL PRACTICE**

**– A B S T R A C T –**

Scientific Coordinator  
**PROFESSOR RAMONA AMINA POPOVICI**

**Timișoara  
2022**

# CONTENTS

List of scientific published articles .....	V
List of abbreviations and symbols .....	VI
List of figures .....	VII
List of tables .....	XI
Dedication .....	XII
Acknowledgment .....	Xiii
INTRODUCTION .....	XV
 <b>GENERAL PART</b> .....	 1
CHAPTER 1. MATERIALS FREQUENTLY USED IN DENTAL PRACTICE .....	1
1.1 Synthetic compounds as antiseptics and disinfectants .....	1
1.2 Products of plant origin .....	4
1.3 Nanomaterials .....	7
CHAPTER 2. EXPERIMENTAL TESTS FOR THE EVALUATION OF DENTAL MATERIALS .....	9
2.1 Introduction .....	9
2.2 Biological <i>in vitro</i> tests .....	13
2.3 Biological <i>in vivo</i> tests .....	22
CHAPTER 3. ETHICAL RULES APPLIED FOR TESTING DENTAL MATERIALS .....	29
3.1 Legislative and specialized notions .....	29
 <b>SPECIAL PART</b> .....	 33
CHAPTER 4. STUDIES ON THE MECHANICAL AND ADHESIVE PROPERTIES OF NANOCOMPOSITES FOR DENTAL USE .....	34
4.1 The influence of inorganic filler on the mechanical properties of nanocomposites .....	34
4.1.1 Introduction .....	34
4.1.2 Materials and methods .....	37
4.1.3 Results and discussions .....	43
4.1.4 Conclusions .....	50
4.2 <i>In vitro</i> study of the adhesive interface and commercial nanocomposites .....	52
4.2.1 Introduction .....	52
4.2.2 Materials and methods .....	53
4.2.3 Results and discussions .....	61

4.2.4 Conclusions .....	65
CHAPTER 5. CLINICAL-STATISTICAL STUDY REGARDING THE USE OF DENTAL COMPOSITES IN DENTAL PRACTICE .....	66
5.1 Introduction.....	66
5.2 Materials and methods .....	66
5.3 Results and discussions .....	69
5.4 Conclusions .....	77
CHAPTER 6. THE ROLE OF POLYMERIC BIOMATERIALS IN DENTISTRY .....	78
6.1 Introduction.....	78
6.2 Materials and methods .....	80
6.2.1 Therapeutic alternative .....	80
6.2.2 Synthesis pathway .....	80
6.2.3 Sample evaluation .....	83
6.2.4 Statistical analysis.....	86
6.3 Results and discussions .....	87
6.3.1 pH evaluation.....	87
6.3.2 Particles size and stability .....	88
6.3.3 <i>In vitro</i> assessments of particles .....	89
6.4 Conclusions .....	97
GENERAL CONCLUSIONS AND CONTRIBUTIONS.....	98
REFERENCES.....	102

## **ABSTRACT**

Oral health is an integral part of general human health and contributes in a definite way to increasing the quality of life. Dental caries and gingivitis are diseases triggered by microbial factors, correlated with both genetic and dietary factors (cariogenic diet). Therefore, the prevention and treatment of carious lesions, especially in the early stages, along with oral health awareness programs, are particularly important for clinicians.

Biomaterials are synthetic substitutes for ordinary materials or materials whose functions have been altered and which are in continuous or intermittent contact with body tissue or body fluids. Exposure to fluids in the oral cavity, caused by the fact that the biomaterial is placed inside the body, imposes some strict restrictions so that various materials can be used as biomaterials. First of all, a biomaterial must be biocompatible - it should not cause a negative response from the body and vice versa. In addition, it should be non-toxic and non-carcinogenic. Biomaterials should have adequate physical and mechanical properties to serve as a replacement for body tissues. For practical use, a biomaterial should be easy to process into various shapes, relatively low cost, and readily available. The biomaterial must have the following properties: a suitable, biocompatible chemical composition, which avoids adverse tissue reactions; excellent resistance to degradation; acceptable resistance to resist masticatory forces; a reduced modulus to minimize bone resorption; high wear resistance to minimize wear and tear. In the oral cavity, both the tooth and the supporting gingival tissue can be easily destroyed by bacterial disease. Dental caries accompanied by the destruction of dental hard tissue (cavities) due to demineralization and dissolution of teeth associated with plaque metabolic activity (a film of mucus that traps bacteria on the surface of the teeth), can cause extensive tooth loss. Whole teeth as well as tooth segments can be replaced or restored using a variety of materials.

In the past, a number of ethical concerns have been raised about the use of biomaterials made from artificial substances, including metals, polymers and ceramics, given that most of them are related to safety and potentially harmful effects on the human body. Nanomaterials are products that have caught the attention of researchers in various fields of medicine in the last two decades. In most cases, researchers aim to develop the structure-property relationships of materials in

order to obtain superior biological activity accompanied by reduced side effects. Improving the biocompatibility of dental materials is necessary and should be based on well-documented tests. Research on material / tissue interactions needs to be constantly developed and adapted, including mechanistic approaches, as this strategy leads to the development of new and more biocompatible materials. Laboratory testing of the mechanical and adhesion properties of nanocomposites, as well as the evaluation of cytotoxic effects of polymeric biomaterials, is an essential step before their clinical use. Despite all the limitations inherent in simulating the oral environment and the different standards used by different manufacturers, it is necessary to find as many relevant test models as possible, taking into account the various interrelated physical parameters in the behavior of the material. Thus, a strategy to improve the administrative and technical conditions for the certification processes of materials, such as the development of *in vitro* tests with increased predictability of data generated for clinical use, but also the investigation of dentists' opinions on their current use as materials for both temporary and permanent teeth, I considered it of interest, being motivated to approach this research topic. In most cases, researchers aim to develop the structure-property relationships of materials in order to obtain superior biological activity accompanied by reduced side effects. Improving the biocompatibility of dental materials is necessary and should be based on well-documented tests. Research on material / tissue interactions needs to be constantly developed and adapted, including mechanistic approaches, as this strategy leads to the development of new and more biocompatible materials.

The purpose of this paper was to study the biomaterials used in dentistry. The research had three major objectives. The first objective is the contributions made in the field of composite nanomaterials for dental use. The second objective was to centralize data from dental practice on the frequency of use of dental composites. The last objective focused on polymeric biomaterials - microparticles for dental use of interest.

**The general part**, comprises three main chapters. The first chapter deals with materials commonly used in dental practice - synthetic compounds such as antiseptics and disinfectants, plant products, nanomaterials and other materials; the second presents experimental tests for the evaluation of dental materials - physico-

chemical, biological tests *in vitro* and *in vivo*; and in the third chapter are presented the rules of ethics applied to the testing of dental materials.

**The special section** covers three main areas: (1) nanomaterials - address two relevant issues, namely the influence of filler on the properties of nanocomposites - being evaluated in terms of specific properties of two nanocomposites obtained in the laboratory compared to a type of commercial nanocomposite and the study *in vitro* of the adhesive structure and interface of commercial nanocomposites, (2) statistical data obtained from the questioning of specialists in the intensity of the use of dental composites, and (3) the role of polymeric biomaterials in dentistry - aimed at obtaining, characterizing and evaluating polymeric biomaterials based on PLGA (co-glycolic polylactic acid) and PU (polyurethanes) of interest in dental practice.

Nanotechnology is a thorough engineering science that through proper design, specific functions and performance leads to obtaining finished products on a scale of less than 100 nm, characterized and controlled at the atomic or molecular level. In the nano dimension, the properties (physical, chemical and biological) of the products differ from the individual atomic / molecular properties and / or bulk matter. The term nanotechnology was invented in 1974, but the concept was established about two decades ago and nanomaterials research was stimulated in the early 1990s with the introduction of the concept of nanotubes. Composite restorative materials were originally developed to overcome the disadvantages of silicate cements and unfilled resins based on methyl methacrylate monomer and its polymer. In addition, aesthetic reasons and concerns associated with amalgam toxicity have propelled them as modern biomaterials in the dental industry. The present research had two main objectives. *The first objective* was to evaluate the bending strength (FS), the compressive strength (CS), the diametric compression DTS (tensile strength) and the Vickers hardness for two experimental nanocomposites AD1 and AD3 compared to the commercial product of Premise <sup>TM</sup> (Kerr company). Research has shown that the mechanical properties of nanocomposites are influenced by the degree and type of inorganic and silane filler and the correct distribution of nanoparticles in the organic phase. The two experimental composites AD1 and AD3 have similar characteristics to those of the Premise <sup>TM</sup> composite, comply with the generally accepted rules and allow the use of these materials for direct coronary restorations, being biocompatible with dental hard tissues. The bending

strength of the AD1 composite has an intermediate value, between the lowest of the AD3 composite and the highest of the Premise™ composite, the differences being statistically significant both between the experimental materials and the commercial material used as a control. The compressive strength of AD3 is significantly higher than that of AD1 and Premise™, due to the aluminum oxide in the AD3 nanofill. The Vickers micro-hardness of the Premise™ composite is significantly higher than that of the AD1 and AD3 experimental composites. The two experimental composite fillers tested AD1 and AD3 have good mechanical properties, similar to those of the Premise™ composite (Figures 1 and 2).

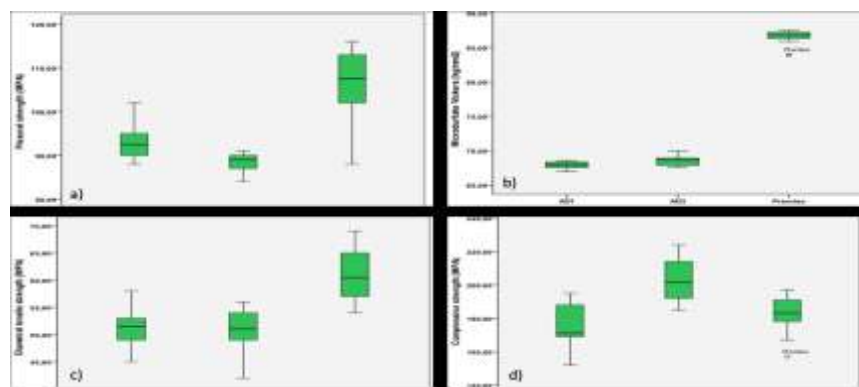


Figure 1 Graphic representation of the results (median, percentage 25-75%, a) bending; b) compression; c) diametrical compressive strength; d) Vickers microhardness

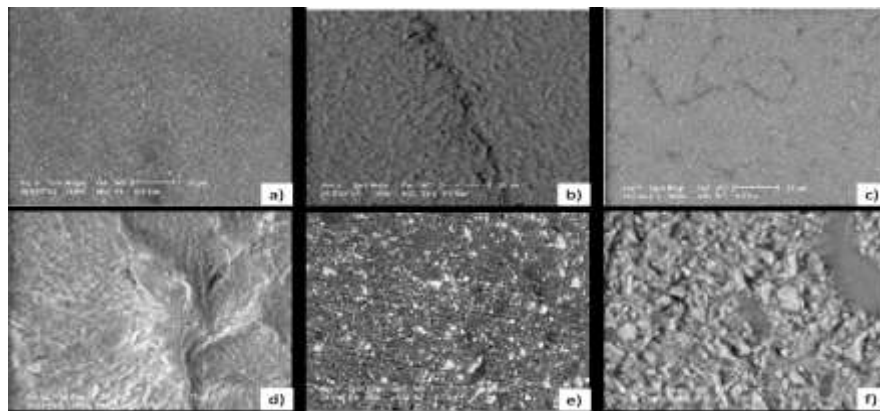


Figure 2 SEM micrographs of tested nanocomposites (1000x): Premises before (a) and after fracturing (b); AD1 before (c) and after fracturing (d); AD3 before and after fracture (f)

*The second objective* was to evaluate the structural characteristics of two commercial nanocomposite materials (Premise™ / Kerr Corp and Tetric EvoCeram / Ivoclar-Vivadent) and to evaluate the marginal fit and the adhesive interface made with two commercial adhesive systems (Optibond SoloPlus™ / Kerr Corp and G-Bond™ / GC). The study showed that the Premise and Tetric EvoCeram

nanocomposites have structural homogeneity and the characteristics of inorganic filler with prepolymerized particles and barium borosilicate fillers with a medium submicron size. The studied nanocomposites have a variable number of porosities and air bubbles, which will cause secondary caries by microinfiltration process. The adhesive systems of various generations used produce a continuous interface and a good marginal adaptation in most cases, being compatible with the tissues in the oral cavity. The most homogeneous and thick hybrid layer is generated by the Optibond Solo Plus adhesive system, but the tightest interface belongs to the specimens in which the G-Bond adhesive was used.

At present there are a multitude of materials used for coronary filling: composites, glass ionomer cements, amalgams, etc. Specialists claim that there is no type of biomaterial used for direct coronary reconstitution that is the best, it is chosen according to the particularities of the clinical case, the operator's preferences, possible allergies of the patient, etc. The main purpose of this study was to investigate the views and current use of biomaterials used in dental treatments for both temporary and permanent teeth. Therefore, the following was carried out: (a) the investigation of the opinion on the use of filling biomaterials for temporary and permanent teeth; (b) the investigation of the opinion on the use of indirect styling materials before the placement of composite restorations by dentists; (c) an assessment of how the level of clinical experience (years of graduation) or postgraduate training influenced their choices. It was observed that the glass ionomer cements, followed by the composite materials were the first choices of dentists for direct dental restorations in the primary dentition. Compomers are an effective alternative to other temporary tooth restoration materials, and yet no questionnaire respondents use this material. This class of biomaterials is not well known among dental practitioners. For filling permanent teeth, nanohybrid composites are preferred to microhybrid ones. Most of the answers were in favor of placing a straight hairstyle in the deep cavities. Making direct styling under composite restorations in moderately deep and deep cavities is controversial among dentists. The current existence on the market of dental products of a multitude of biomaterials for the restoration of teeth makes it more difficult to choose the right material.

Research in the field of materials science and engineering has expanded greatly in recent decades, especially in the field of biocompatible materials, known



as biomaterials. There are two main reasons for this progress: on the one hand, medicine is constantly looking for solutions to many health problems, and on the other hand, certain classes of materials have already proved useful in alleviating or even curing certain human suffering. The use of polymeric materials has increased in dentistry, not only due to their excellent surfaces, but also due to their excellent mechanical and biological properties, as well as the low cost of production and ease of processing. The aim of this study was the analysis of polymeric materials (of interest in dentistry), and the evaluation of biocompatibility on human primary gingival fibroblasts - HGF cells, in terms of cell viability and cytotoxicity. In addition, the behavior of keratinocytes and squamous cell carcinoma cells in the presence of the obtained biomaterials was verified. Microparticles with an average size between 160 and 200 nm (Table 1) were obtained which have a non-cytotoxic potential, evaluated by specific viability tests (MTT test) and cytotoxicity (LDH test) on primary human gingival fibroblasts.

**Table 1 Characteristics of synthesized microparticles**

Sample	Particle size (nm)		Zeta Potential (mV) Mean $\pm$ SD
	Mean $\pm$ SD	PDI	
PU_1	161 $\pm$ 11	0.5	25.3 $\pm$ 3.5
PU_2	127 $\pm$ 19	0.6	29.8 $\pm$ 2.9
PU_3	183 $\pm$ 7	0.6	26.4 $\pm$ 2.1
PU_4	152 $\pm$ 13	0.6	24.9 $\pm$ 3.1
PLGA	192 $\pm$ 8	0.5	32.1 $\pm$ 1.9

The synthesized products did not show a significant inhibitory potential on the viability of fibroblasts, this being a first confirmation that they can be used in the oral cavity. Although the biomechanical properties of polymeric materials are dictated by their bulk properties, their interactions with tissues are governed by their surface properties, which can be easily adapted to specific requirements.

**Own contributions** are: (1) on the influence of inorganic particles by their size, type and distribution on the mechanical and adhesive properties of composite materials; (2) related to the clinical-statistical study on the use of dental composites in dental practice using the questionnaire survey, the questionnaire is original and designed to appreciate, explore and know the facilitation and experience of the use

of direct restorative materials by dentists (distributed by google forms); (3) regarding polymeric biomaterials with an average size between 160 and 200 nm, they have no cytotoxic potential, and MTT and LDH tests on primary human gingival fibroblasts show that the synthesized products did not show a significant inhibitory potential on fibroblast viability.

**Future research directions** should include: studies on the mechanical properties of biomaterials: tensile strength, compression and bending; studies on water absorption by dental biomaterials; studies on the clinical behavior over time of some dental biomaterials (postoperative sensitivity; ability to match colors; marginal integrity; surface texture; marginal dyschromia; appearance; abrasion).