Comparative Study of Cytotoxicity of Newly Developed Calcium Phosphate-Based Root Canal Sealers by MTT Assay

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Dedication

This piece of work is gratefully devoted

To my precious parents,

To my supporting brother ,

Special thanks to my wife.

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First and for the most, thanks to **ALLAH** the most Gracious and most merciful.

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Introduction

Cytotoxicity is the degree to which an agent possesses a specific destructive action on certain cells or the possession . Cytotoxicity testing is performed in compliance with ISO 10993-05 and USP <87> using Baby hamster kidney fibroblastic cells (BHK-21). Researchers use this service to release raw materials.

A cytotoxicity test determines whether a product or compound will have any toxic effect due to leachable on living cells. Generally used as a screening tool for raw materials or component products before they are put into the design of a medical device.

Various methods proposed for assessing the cytotoxicity of root canal cement. Biological compatibility of root canal sealers is of importance as these materials encounter periapical tissues including fibroblasts. The tissue response to these materials may influence the outcome of root canal treatment.

Continuing advances usage of bioactive root canal sealers that contains calcium phosphate has promising results. Physical and mechanical properties of experimentally synthesized Calcium phosphate cement (CPC) recommended its use as root canal cement. To justify its clinical use cytotoxicity to living cells has to be assessed.

Review of Literature

Several classes of endodontic sealers are currently used in clinical practice, but all have substantial limitations. Zinc oxide-eugenol-based endodontic sealers have been used for many years, but release potentially cytotoxic concentrations of eugenol. Calcium hydroxide-based sealers promote calcification but tend to dissolve overtime and compromise the endodontic seal. Glass–ionomer sealers may bond tooth structure but also may activate the release of prostaglandins in periapical tissues. Resin based sealers are increasingly gaining despite their well-documented toxicity and mutagenicity. Being based on the principal component of human dentin, calcium phosphate cements are promising when used as root canal sealers.

I. Physical and chemical properties of calcium phosphate cements:

The properties of a dental material affect its biocompatibility therefore any change in its properties will be reflected on its behavior in the periapical tissue.

El Briak et al [(1)] (2002) studied physical properties of calcium phosphate cement, such as initial and final setting times, cohesion time, dimensional and thermal behavior by varying different parameters such as liquid to powder (L/P) ratio, molar calcium to phosphate (Ca/P) ratio and the pH and the concentration of the sodium phosphate buffer. The best results were obtained with the pH 7 sodium phosphate buffer at the concentration of 0.75 M. With this liquid phase, physical properties depended on the Ca/P and L/P ratios, varying from 6 to 10 min (initial setting time), 11 to 15 min (final setting

time). This cement expanded during its setting (1.2–5 % according to Ca/P and L/P ratios); this would allow a tight filling.

Takagi et al [2] (2003) investigated the properties of water-free, glycerol-containing calcium phosphate cement (CPC) pastes that are stable in the package and would harden only after being delivered to a defect site where glycerol–tissue fluids exchange occurs. Premixed CPC pastes were prepared by combining cement liquids containing glycerol and various amounts of hydroxypropyl methylcellulose, with CPC powder. The hardening times were measured on samples that hardened in a model that allowed exchange of glycerol and physiologic-like solution (PLS) through fritted glass slides at 37 °C. All pastes had excellent washout resistance; they remained intact and hardened while immersed in PLS and formed HA as products.

Komath andVarma [3] (2003) modified a conventional two-component calcium phosphate cement formulation with a biocompatible gelling agent to induce flow properties and cohesion. A powder part contained dry mixture of acidic and basic calcium phosphate particles and a liquid part containing phosphate solution. The quantity of the gelling agent was optimized to get a viscous paste, which was smoothly injectable through an 18-gauge needle, with clinically relevant setting parameters. The new formulation had setting time of 20 minutes and a compressive strength of 11 MPa. Fourier transform infrared spectrometry, and energy dispersive electron microprobe analyses showed that the ingredients of the cement were converted to hydroxyapatite. Scanning electron microscopy revealed a porous structure with particle sizes of a few micrometers. The cement did not show any appreciable dimensional or thermal change during setting.

Takagia et al [4] (2003) evaluated chitosan as the matrix for preparing calcium phosphate (CPC)-chitosan composites. Cement specimens were prepared by mixing CPC powder, which is an equimolar mixture of tetracalcium phosphate and dicalcium phosphate anhydrous with a chitosan solution at a powder/liquid ratio of 2–2.5. The setting time was measured by a Gilmore needle method. Powder X-ray diffraction analysis was used to determine the conversion of the CPC to hydroxyapatite. The CPC–chitosan composites were more stable in water than conventional CPC. They did not disintegrate even when placed in water immediately after mixing. The CPC–chitosan paste hardened within 10 min in all cases. Chitosan did not interfere the conversion of CPC components to hydroxyapatite.

Bigi et al [5] (2004) investigated the effect of gelatin on the setting time, compressive strength, phase evolution and microstructure of calcium phosphate cement. The composite cement powder (18wt% gelatin, and 82 wt% α-tricalcium phosphate) was prepared from the solid compound obtained by casting a gelatin aqueous solution containing α -tricalcium phosphate. 5wt% of calcium hydrogen phosphate dihydrate were added to the powder before mixing with the liquid phase. Two cement formulations were prepared using two different liquid/powder ratios, and their properties were compared with those of control samples prepared without gelatin. The final setting time increases from 10 minutes to more than 45 minutes when the L/P ratio increases from 0.3 to 0.4 ml/g. The presence of gelatin accelerates the setting reaction, and improves the mechanical properties of the cements.

Ginebra et al [6] (2004) investigated the possibility of controlling the final micro and nano-structural features of a calcium phosphate cement by modifying the particle size of the starting powder, and to study the effect of

this parameter on the kinetics of the setting reaction. The development of calcium phosphate materials with tailored structures at the micro and nanoscale levels could allow the modulation of some specific responses in biologic phenomena such as protein adsorption and cell adhesion, which strongly depend on the nano-sized roughness of the interface. They showed that, the higher specific surface, produced by the reduction of the particle size of the powder, strongly accelerates the hydrolysis of the α-TCP into calcium-deficient hydroxyapatite. The higher degree of super-saturation attained in the solution favors the nucleation of smaller crystals. The reduction of the particle size produces a substantial decrease of the setting time and accelerates the hardening of the cement without significantly affecting the final strength attained.

Liu et al [(7)] (2006) investigated the steady and dynamic rheological properties of concentrated aqueous injectable calcium phosphate cement (CPC) slurry. The concentrated aqueous injectable CPC showed both plastic and thixotropic behavior. As the setting process progressed, the yield stress of CPC slurry was raised, the area of the thixotropic hysteresis loop was enlarged, indicated that the strength of the net structure of the slurry had increased. Dynamic rheological behavior indicated that the slurry presented the structure similar to viscoelastic body and the property of shear thinning at the beginning. During the setting process, the slurry was transformed from a flocculent structure to a net structure, and the strength increased. Different factors had a diverse effect on the rheological properties of the CPC slurry in the setting process, a reflection of the flowing properties, and the microstructure development of this concentrated suspension. Raising the powder-to-liquid ratio decreased the distance among the particles, increased the initial strength, and shortened the setting time.Raising the temperature

improved the initial strength, increased the order of reaction, and shortened the setting time, which was favorable to the setting process. The particle size of the raw material had much to do with the strength of original structure and setting time.

Julien et al [8] (2007) investigated amorphous CPC cements doped with various ions Mg, Zn and F in an attempt to improve their physicochemical and biological properties. The cement samples were composed of a settable matrix and biphasic calcium phosphates (BCP) granules. X-ray diffraction data of the cement matrices after 24 hour setting revealed apatite with poor crystalinity, whereas BCP granules did not react. Scanning electron microscopy (SEM) revealed that the crystals produced after setting reaction were poorly crystalline consisting of petal-like crystals. Those of Mg doped samples look smaller and finer. SEM observations and measurement of mitochondrial activity (MTT assay) indicated that the MC3T3-E1 cells remained viable in contact with the various materials. In addition, the results indicated that MC3T3-E1 cells maintained their capability to express alkaline phosphatase on the various materials.

Hockin et al [9] (2007) formulated premixed CPC (PCPC) with rapid setting, high strength, and good in vitro cell viability. PCPCs were formulated from CPC powder, non-aqueous liquid, gelling agent, and hardening accelerator. Five PCPCs were thus developed: PCPC-Tartaric, PCPC-Malonic, PCPC-Citric, PCPC-Glycolic, and PCPC-Malic. Formulations and controls were compared for setting time, diametral tensile strength. All the new PCPCs with various organic acids as hardening accelerators hardened much faster than the Premixed Control. The diametric tensile strength results showed that most cement showed moderate strength

increases upon increasing the immersion time. Between different materials, the strength of PCPC-Tartaric was not significantly different from that of PCPC-Malonic and PCPC-Malic. The strength of PCPC-Tartaric was significantly higher than PCPC-Citric, PCPC-Glycolic and the premixed control.

Burguera et al [10] (2008) evaluated the effect of the calcium to phosphate ratio on a tetracalcium phosphate(TTCP)-dicalcium phosphate dihydrate (DCPD) cement. Six groups of different Ca/P ratio were developed. The resulting six TTCP-DCPD cement mixtures were characterized using X-ray diffraction analysis, scanning electron microscopy, and pH measurements. Setting times measured. Setting times was longer when water was the cement liquid than when sodium phosphate solution was used, and the calcium to phosphate ratio did not have a marked effect on this property.

Asgary et al [11] (2008) analyzed the physical properties of a new experimental cement (NEC) and compare them with those of mineral trioxide aggregate (MTA). Those were pH, setting time, flow and film thickness of NEC and MTA assessed. NEC consisting of different calcium compounds (i.e, calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide, and calcium chloride) was developed. For chemical compositions, all specimens imaged and analyzed by scanning electron microscopy and electron probe microanalysis (EPMA). pH of NEC and MTA showed similar results. Shorter setting time obtained with the NEC compared with MTA.

Ghazvini et al [12] (2009) measured and compared the pH and phosphate and calcium ions release of a new endodontic material calcium enriched