



α -Tricalcium phosphate/fluorapatite-based cement – Promising dental root canal filling material

Abdul Kazuz¹, Željko Radovanović^{2,*}, Djordje Veljović¹, Vesna Kojić³, Dimitar Jakimov³, Tamara Vlajić-Tovilović⁴, Vesna Miletić⁵, Rada Petrović¹, Djordje Janačković¹

¹University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia

²University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia

³University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia

⁴University of Belgrade, School of Dental Medicine, DentalNet Research Group, Belgrade, Serbia

⁵University of Sydney, Sydney Dental School, Camperdown, Sydney, Australia

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Abstract

α -Tricalcium phosphate/fluorapatite (α -TCP/FAP) mixed powders were prepared in order to obtain dental root canal filling cements. Different liquids for cement pastes have been investigated and the most suitable one for obtaining rheologically optimal pastes was chosen for further analysis. Morphological changes in the cement materials as a consequence of the formation of hydroxyapatite (HAp) after the immersion in a simulated body fluid (SBF), an influence on the cell viability, and final success of the filling were investigated by field emission scanning electron microscopy. Treatment of the α -TCP/FAP mixtures in SBF at 37 °C resulted in a complete transformation of α -TCP into HAp after 10 days, while the exposure of MRC-5 human and L929 animal fibroblast cells to the cement showed complete absence of cytotoxicity. The root canal of an extracted tooth was filled with the α -TCP/FAP cement containing 5 wt.% of FAP and relatively strong adhesion between the cement and dentine was observed after 48 h. The same cement material was immersed during 10 days in SBF and after that both human and animal fibroblast cells during in vitro MTT tests showed higher cell viability compared to the control sample. These findings lead to a conclusion that the α -TCP/FAP based cement demonstrates potential for further development towards dental cement application.

Keywords: apatite, dental cement, bioactivity, biocompatibility, dental materials

I. Introduction

The most important feature of biomaterials is their biocompatibility, ability to be incorporated into a living organism without toxic effect on the body of the host. Also, biomaterials must have good mechanical and chemical properties, in order to withstand a load they are exposed to, and to avoid any undesirable reaction with the environment, while retaining bioactivity to ensure healthy tissue growth. They have found application in cardiovascular surgery (heart valves, vascular grafts), dentistry (tooth filling, dental crowns and implants) and dermatology (artificial skin) [1–3]. Calcium phosphates (CaPs) are considered as excellent biomaterials due to

their similarity to teeth and bones, opening the possibility for the synthesis, investigation and modification of these materials, in order to achieve desired properties [2,4,5].

Osteoconductivity is another valuable property of CaPs, making them ideal candidates for scaffolds [6,7], and CaPs can also be used as drug carriers [8–10]. The most commonly used CaPs are hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAp), α - and β -tricalcium phosphate (TCP) [5,11–13]. In order to increase biocompatibility and mechanical properties, HAp, α - and β -TCP have already been modified using various doping elements and sintering conditions [14–17].

HAp with a Ca/P ratio of 1.67, among others CaPs, shows great stability under physiological conditions [5]. Replacement of the OH^- group in HAp with an F^- ion yields fluorapatite (FAP), the most stable CaPs [5]. Fluorine is an important element in the tooth and deposits on the

*Corresponding author: tel: +381 11 3303740,
e-mail: zradovanovic@tmf.bg.ac.rs

enamel in the form of FAp slowing down demineralization [18,19]. This feature makes fluoride-containing bioceramics valuable in the design of restorative materials for dental applications. FAp could also represent a very useful component of ceramic-based composite materials, increasing the biocompatibility and mechanical strength of a matrix [2,20,21].

Materials for the tooth filling must achieve efficient and long-term adhesion to tooth tissues, enamel and dentine, in order to avoid the plaque accumulation, bacterial invasion and the formation of secondary caries. Cements used in dental application must meet requirement of the suitable setting time, to ensure that dentist/surgeon has enough time to finish the procedure [22]. Besides setting time, cement must fulfil working time, flow and solubility, all according to standard ISO 6876 – Dental root canal sealing materials [23]. Moreover, other more accessible locations may be of interest as potential indications for this biocompatible and bioactive α -TCP/FAp cement, such as temporization in high caries-risk patients as part of minimal intervention dentistry approach [24], temporization and/or lining in deep cavities following selective caries removal to caries-affected dentine [25]. Partially demineralized dentine may benefit from F^- ion interaction between the cement and dentine hydroxyapatite as well as the formation of new apatite crystals due to the bioactive nature of the cement. Experimental calcium silicate cements have shown the potential to remineralize caries-affected dentine [26].

In our previous research, we showed the beneficial effect of combining FAp with α -TCP on the improvement of compressive strength and biocompatibility [27]. In this study, we investigate the impact of different liquid components on the rheology of dental root canal filling paste, as well as the influence of a new liquid phase in composite on the bioactivity of α -TCP/FAp based cement after exposure to a simulated body fluid (SBF). The new liquid phase in the composite significantly improves the rheological properties of the paste compared to the paste obtained in the previous work [27] and thus this material is much more suitable for the role of dental root canal filler, which was our primary goal. *In vitro* MTT and DET tests were performed on MRC-5 and L929 cells to investigate the biocompatibility of the potential dental root filling material. Field emission scanning electron microscopy (FESEM) was used for observation of tooth filling success, as well as for assessment of MRC-5 and L929 cells seeded on the cement surface. It has been observed that the obtained material has satisfactory rheological properties within the dental standards and needs.

II. Experimental procedure

2.1. HAp, α -TCP and FAp preparation

For HAP and FAp powders synthesis, calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, $\geq 98\%$, Carl Roth,

Germany), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, 99.8%, VWR), sodium fluoride (NaF, 99%, Riedel-de Haën, Seelze-Hannover, Germany) ammonium hydroxide (NH_4OH , p.a., Zorka Pharma, Serbia) and ethylenediaminetetraacetic acid disodium salt dehydrate ($Na_2H_2EDTA \cdot 2H_2O$, CIII; Sigma-Aldrich, USA) were used, as in our previous research [27].

HAp powder was synthesized via the hydrothermal method, with Ca/P molar ratio of 1.50, the same as in our previously published studies [27,28]. α -TCP powder was obtained by calcination of the HAp at 1500 °C for 2 h. FAp powder was obtained in a similar way as the HAp, with the addition of F^- ion source [27].

2.2. Tooth filling cement preparation

Composite cement pastes were made of α -TCP/FAp mixed powder with 5 wt.% of FAp and different liquids in order to obtain pastes with the best rheological properties. Based on the literature data, citric acid ($HOC(CH_2CO_2H)_2$, CA, p.a., Zorka Pharma Serbia), disodium hydrogen phosphate (Na_2HPO_4 , p.a., Merck, Germany), polyethylene glycol ($C_{2n}H_{4n+2}O_{n+1}$, PEG, Sigma-Aldrich, USA) and hydroxypropyl methylcellulose ($C_{56}H_{108}O_{30}$, HPMC, Sigma-Aldrich, USA) were selected for the preparation of the water-based liquid phase [22,28–31]. However, they were mixed with water under different wt.% ratio in order to optimize the composition of liquid for the powder mixture. Also, by varying the liquid-to-powder ratio (LPR) from 0.3 to 0.5 ml/g, with a step of 0.01, the best LPR ratio was determined. Five series of samples were prepared, F1–F5, with different compositions of the liquid phase and LPR (Table 1).

SBF, prepared as per the Kokubo *et al.* formulation [32], was used for the *in vitro* investigation of bioactivity. The α -TCP/FAp samples with 0 (as a control), 5 and 10 wt.% of FAp were kept in SBF for 10 days. The samples were labelled as α -TCP/FAp(0), α -TCP/FAp(5) and α -TCP/FAp(10), respectively. The same prepared samples were used for *in vivo* MTT and DET biocompatibility tests.

2.3. Characterization

The values of solubility, flow, working and setting time were determined in accordance with standard ISO 6876 – Dental root canal sealing materials [23].

X-ray powder diffraction (XRPD) was performed using a Rigaku Smart Lab diffractometer with CuK_α radiation in the 2θ angle range from 20 to 60° with a step scan of 0.02° and the speed of 10°/min. Morphological changes of cement after the exposure to SBF, the biocompatibility of composites and tooth filling were investigated using FESEM (Tescan Mira 3 XMU) on gold-coated samples using the Polaron SC502 sputter coater. Cytotoxicity was investigated via *in vitro* MTT ($N = 3$ group⁻¹) and *in vitro* DET ($N = 1$) tests 48 h after the diluted MRC-5 and L929 cells seeding in 96-

Table 1. The composition of different water-based liquids for cement pastes and best LPR values

Samples series	Composition of the liquid [wt.%]	LPR [ml/g]
F1	20% CA, 10% PEG	0.32
F2	2.5% Na ₂ HPO ₄	0.32
F3	10% PEG	0.34
F4	10% CA	0.39
F5	0.5% HPMC, 15% CA, 10% PEG	0.45

Table 2. Physical properties of α -TCP and α -TCP/FAp composite cement

Samples	Flow [mm]	Solubility [%]	Working time [s]	Final setting time [min]
α -TCP/FAp(0)	16.3	1.9	270	24
α -TCP/FAp(5)	15.6	1.8	270	22
α -TCP/FAp(10)	16.0	2.2	270	27

well plates; the procedure was the same as in formerly published researches [27,33].

III. Results and discussion

3.1. Physical properties of cement samples

Table 1 presents liquids used for cement pastes preparation and the best values of LPR. CA is often added to the liquids for pastes because it has been reported as a setting accelerator and a calcium ion chelating agent that improves mechanical properties [29,34]. PEG is used for phase stability, i.e. to prevent segregation [30]. Another ingredient added to the solution for rheology

modification is HPMC, because it reduces washability, which prolongs the lifetime of cements [31,35]. All of the ingredients added to the starting solution should ensure better cement paste handling and good mechanical properties. Also, desired paste properties could be achieved by controlling the LPR value.

Due to the high concentration of CA, the setting of the F1 series was too fast and was disregarded for further study. The F2 series, containing Na₂HPO₄ that increases the setting rate, was also deemed unsuitable for a dental cement, as well as the F3 series, in which PEG was used for the enhanced phase stability. The viscosity of the F4 series was too low for further use. The F5 se-

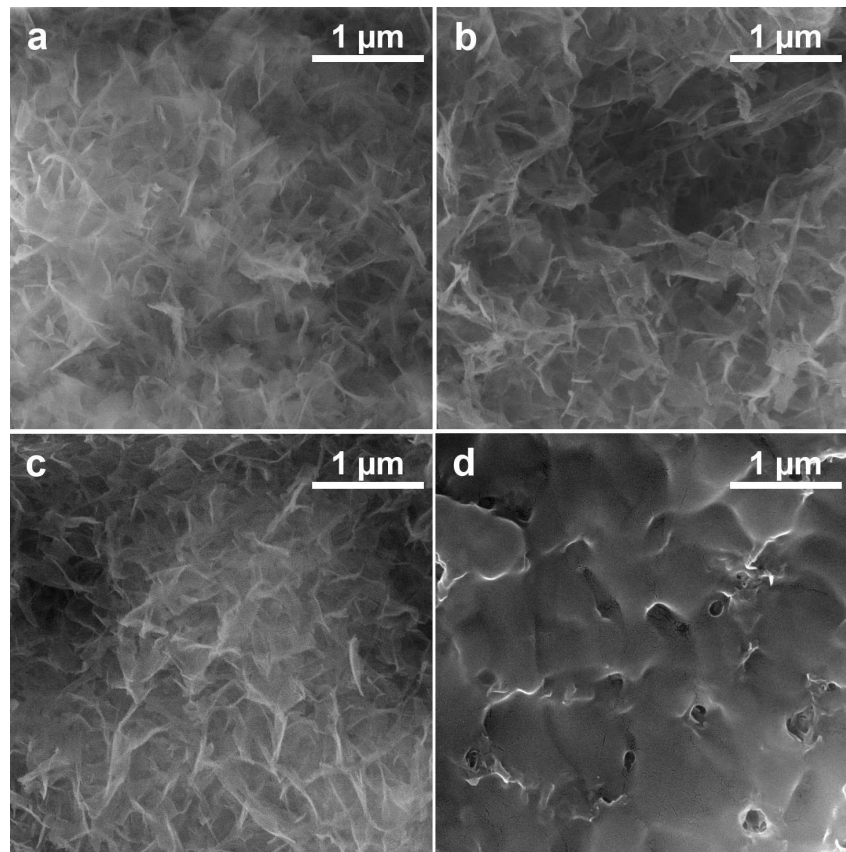


Figure 1. FESEM images of the surface of samples after 10 days in SBF: α -TCP/FAp(0) (a), α -TCP/FAp(5) (b) and α -TCP/FAp(10) (c) and α -TCP/FAp(0) without staying in SBF (d)

ries that contained HPMC, CA and PEG, and with LPR of 0.45 ml/g exhibited the best setting time and was chosen for further analysis with α -TCP/FAp mixed powders.

Table 2 presents flow, solubility, working and setting time for the samples of F5 series: pure α -TCP and α -TCP/FAp with 5 and 10 wt.% of FAp that were labelled as α -TCP/FAp(0), α -TCP/FAp(5) and α -TCP/FAp(10), respectively. The flow of all samples was slightly short of the minimum of 17 mm, required by ISO 6876, probably due to a strong cohesion induced by the addition of PEG [36,37]. Solubility was in agreement with the requirements of ISO 6876 (<3 wt.% mass fraction) and similar to other tooth filling materials found in the literature [38,39]. Working time is below the upper limit determined by ISO 6876 (30 min). Setting time is lower compared to pastes with HPMC and the LPR value of 0.45 ml/g is comparable, presumably as a result of a high concentration of CA [29,40]. Both α -TCP/FAp based pastes have potential as new filling materials, but α -TCP/FAp(5) could be more appropriate due to the shorter setting time.

3.2. Analyses of composite cements bioactivity

The most commonly used *in vitro* method for the evaluation of bioactivity of obtained materials is the FESEM observation of the formation of a new HAp layer onto the surface of the tested material in SBF solution.

HAp is a dominant dentine and bone constituent, and its formation on the surface of the material that was laid in SBF solution indicates high biocompatibility of the material [41]. Also, the formation of HAp by the transformation of α -TCP on the surface of α -TCP/FAp composite cements in SBF ensures interaction between the host hard tissue and ceramic biomaterial. Therefore, soaking of the samples in SBF and monitoring formation of the crystals give valuable information about dental material's behaviour in the human body. FESEM images (Figs. 1a-c) present morphological changes of the sample surfaces after 10 days in SBF in comparison with the surface of the sample that did not reside in the SBF (Fig. 1d). HAp layer formation was observed in all samples (Figs. 1a-c) and the new phase becomes dominant over the entire surface [27,41] that could confirm the expected bioactivity of composite cement [42].

The XRPD analyses confirm observation by FESEM and further show that in all samples α -TCP was completely transformed to HAp during soaking in SBF (Fig. 2). Also, the positions of 2θ angles in the pattern of the samples α -TCP/FAp(5) and α -TCP/FAp(10) are slightly shifted to the higher values in comparison to pure α -TCP indicating the presence of FAp with a smaller unit cell. In addition, XRPD patterns of the cement samples that have not been immersed in SBF (Fig. 2) show the partial transformation of α -TCP into HAp after 2 days.

3.3. Analysis of composite cements biocompatibility

Visual investigation of biocompatibility and cytotoxicity of the samples α -TCP/FAp(0), α -TCP/FAp(5), and α -TCP/FAp(10), after 10 days in SBF, and their influence on L929 and MRC-5 fibroblast cells are presented in Figs. 3 and 4. The cell growth on each sample was prominent, indicating no cytotoxicity of α -TCP/FAp cement samples on L929 fibroblast cell culture (Fig. 3) [43].

MRC-5 retained the shape on α -TCP/FAp(0), but it can be also observed on each composite (Fig. 4). In all samples, cytoplasmic extensions were observed, indicating that composite cements are not toxic for fibroblast MRC-5 cells [14].

3.4. *In vitro* MTT and DET assay

Along with FESEM, *in vitro* MTT and DET methods were employed for the investigation of the influence of FAp on the biocompatibility of the samples. Human fibroblast MRC-5 cells and animal fibroblast L929 cells were used as test cultures for viability measurement in the presence of the samples α -TCP/FAp(0), α -TCP/FAp(5), and α -TCP/FAp(10), after 10 days in SBF. Cell viability obtained with MTT after 48 h is presented in Fig. 5.

Compared with the control samples, the α -TCP/FAp(5) and α -TCP/FAp(10) showed an increase in cell viability on MRC-5 and an insignificant drop of cell viability on L929, proving non-cytotoxicity of the prepared composites with FAp. Synthetic HAp was

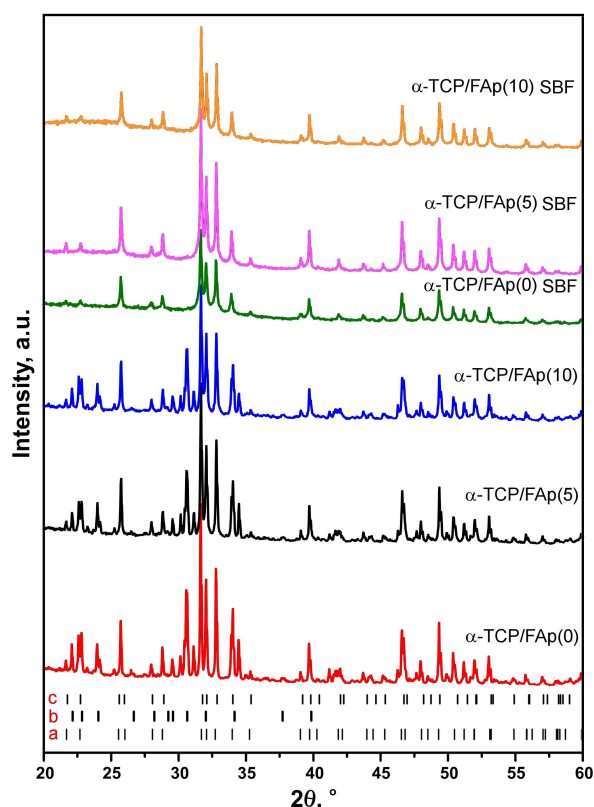


Figure 2. XRPD patterns of samples: α -TCP/FAp(0), α -TCP/FAp(5) and α -TCP/FAp(10), and the same samples after 10 days in SBF (a - HAp - PDF#74-0556, b - α -TCP - PDF#29-0359 and c - FAp - PDF#83-0556)

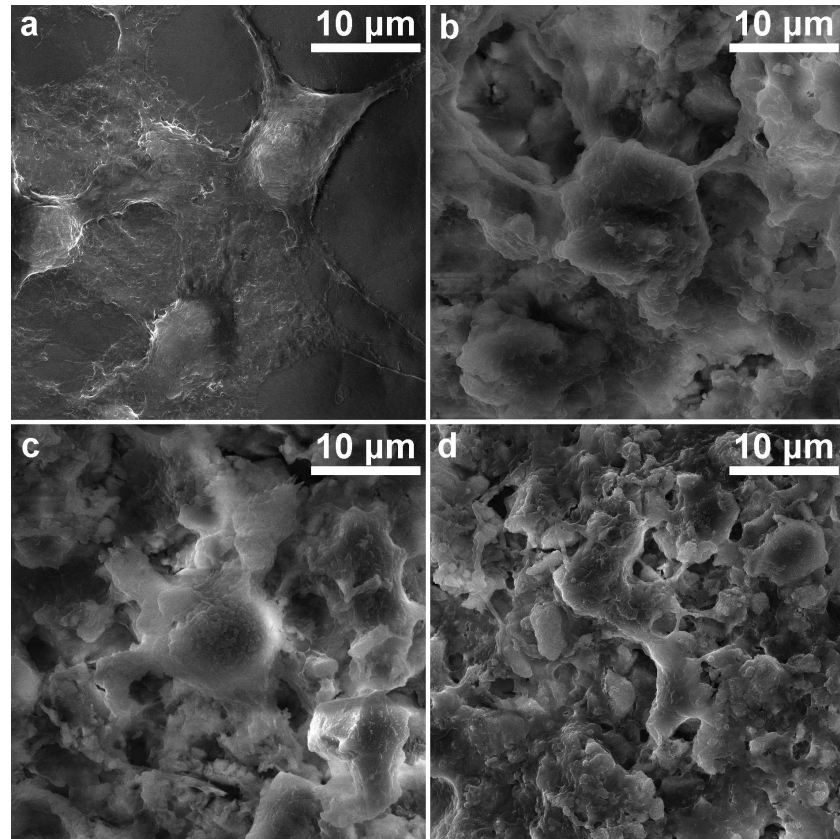


Figure 3. FESEM images of L929 (a) on: α -TCP/FAp(0) (b), α -TCP/FAp(5) (c) and α -TCP/FAp(10) (d)

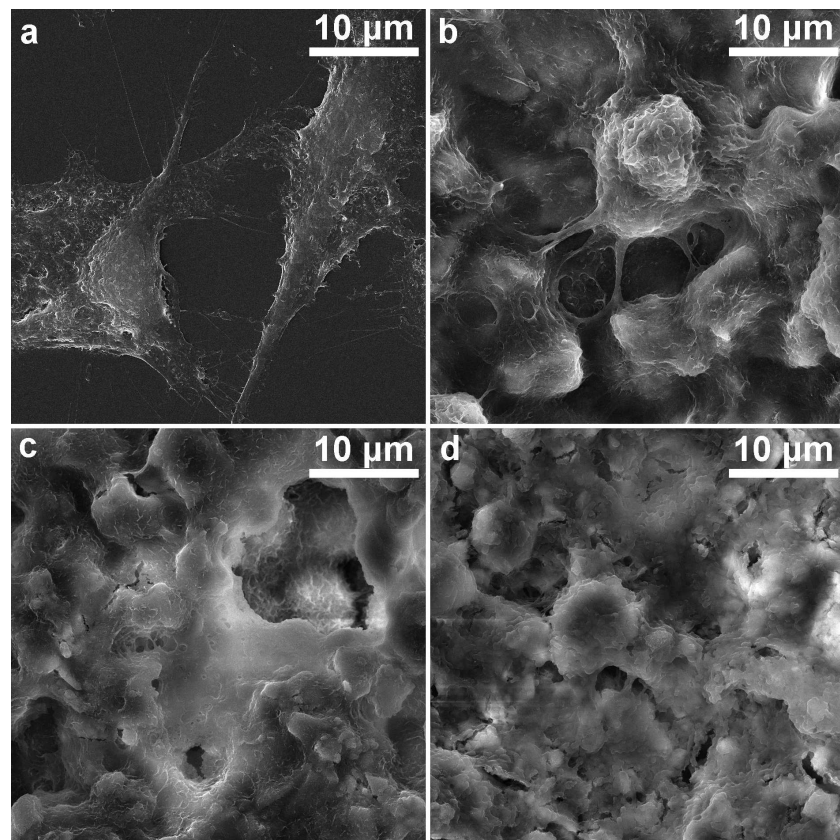


Figure 4. FESEM images of MRC-5 (a) on: α -TCP/FAp(0) (b), α -TCP/FAp(5) (c) and α -TCP/FAp(10) (d)

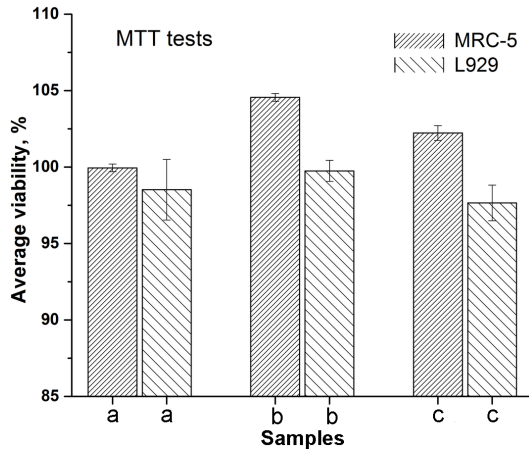


Figure 5. The results of *in vitro* MTT assay on samples: α -TCP/FAp(0) (a), α -TCP/FAp(5) (b), and α -TCP/FAp(10) (c)

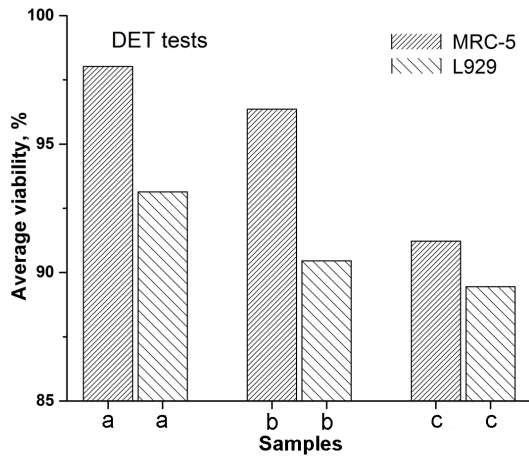


Figure 6. The results of *in vitro* DET assay on samples: α -TCP/FAp(0) (a), α -TCP/FAp(5) (b) and α -TCP/FAp(10) (c)

already confirmed as non-cytotoxic [44–46], although L929 cell viability in the presence of our composites α -TCP/FAp(5) is higher. The sample α -TCP/FAp(0) induced lower viability of MRC-5 compared to the composites α -TCP/FAp(5) and α -TCP/FAp(10), which was in accordance with the literature [47]. As it can be seen from Fig. 5, the cell viability is higher for the sample α -TCP/FAp(5) that has been chosen for the dental root canal filling.

The *in vitro* DET results presented in Fig. 6 are in agreement with the MTT assay – both composites cause a negligible decrease in cell viability of MRC-5 in comparison to α -TCP/FAp(0). The same trend was observed in the case of L929 cells, for which the sample α -TCP/FAp(10) showed the lowest biocompatibility, probably due to the formation of segregates.

3.5. Analysis of dental cement adhesion to the tooth

Based on the investigated biocompatibility, bioactivity and mechanical properties of two α -TCP/FAp composites in this study and our previous research [27], the sample α -TCP/FAp(5) was chosen as the most appropriate of the tested formulations for the tooth canal filling. Figure 7 presents representative cross-sections of teeth whose canals were filled with either α -TCP/FAp(5) cement (Fig. 7a-c) or with a combination of the α -TCP/FAp(5) cement and gutta-percha (Fig. 7d-f). FE-SEM analysis showed areas of microgap formation in both cases between the α -TCP/FAp(5) and dentine after 48 h in SBF, as it was similarly shown by Kim *et al.* [48]. However, the formation of the HAp layer can be observed at the interface, indicating the potential for improved material-dentine adhesion over time. It is still very early to draw a conclusion on the bonding po-

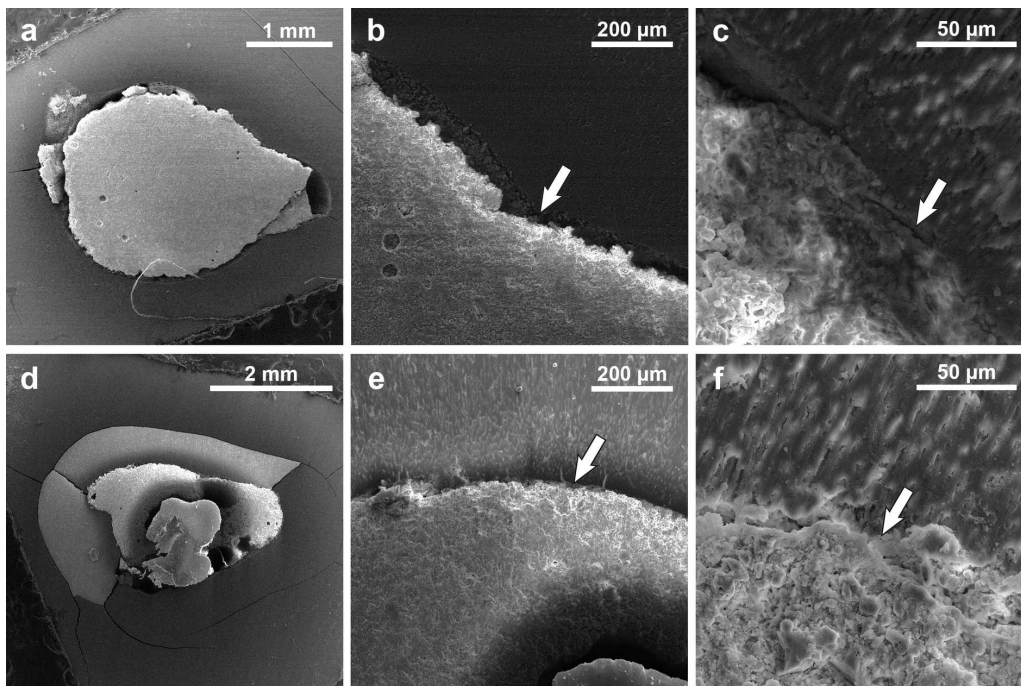


Figure 7. FESEM images of tooth canal filling with α -TCP/FAp(5) without (a-c) and with gutta-percha (d-f) after 48 h in SBF

tential of the tested experimental cement. Further bond strength studies are required over short- and long-term periods on a larger sample size. Yet, the viscosity, working and setting time tested in this study appear suitable for the application of this experimental formulation as a root canal sealer. Potential for α -TCP/FAP cement remains to be experimentally confirmed, but strong bioactive behaviour observed in the present study points in this direction.

IV. Conclusions

This research reported the synthesis and characterization of α -TCP/FAP dental cement material with different concentrations of fluorapatite, as well as different liquid composite-based pastes for dental root canal filling. The best paste with appropriate physical properties was made of α -TCP/FAP(5) that contained 5 wt.% of FAP phase and a liquid containing HPMC, CA and PEG, at a liquid-to-powder ratio of 0.45 ml/g. Bioactivity was confirmed through the transformation of α -TCP into HAP after the immersion in simulated body fluid for 10 days. *In vitro* MTT and DET assays revealed non-cytotoxicity on both human and animal fibroblast cells and guarantee safety for the application of obtained cement in dentistry. It was shown that α -TCP/FAP(5) cement is simple for manipulation and suitable for the filling of the root canals of teeth, which makes this material promising for further development towards dental cement application.

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