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BIOCELLULOSE NANOWHISKERS CEMENT COMPOSITES FOR ENDODONTIC USE

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In this contribution we present the preparation and characterization of biocellulose nanowhiskers–MTA (mineral trioxide aggregates) cement composites for endodontic applications. Nanocrystalline biocellulose fibers were obtain by special procedure starting from bacterial cellulose grown and selected in special conditions. The prepared biocellulose nanowhiskers and commercial MTA cement were used for preparation of some composite: MTA-E (mineral trioxide aggregates-experimental), MTA-10% biocell and MTA-33% biocell cements. Considering one day hardened composites and taking into account XRD patterns and the thermal analysis data, we have concluded that the presence of biocellulose accelerates the hardening processes of MTA cement, decreasing in the same time the quantity of calcium hydroxide crystals. SEM images reveal, for the first time in the literature, crystalline hydrosilicates of high density grown around biocellulose nanowhiskers.The composite biocompatibility was good and was demonstrated by a test with HEp cells in ratio1 mg/ml MTA+33%biocel for 24h. Cells morphology and viability were identically with the ones of the untreated control. Investigations on composites morphology, weight losses, mineralogical composition and structure were performed by SEM, DTA, XRD and TEM respectively.

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1. Introduction

At the end of the 20th century, the use of hydraulic cements in dentistry attracted the interest if the scientific community. Thus, since than, these cements were used in the endodontic, for channel filling or punctured channel repairing [1-10].

The hydraulic cements (hardening processes in presence of water) can be silicate or aluminate and have poor mechanical resistance, but very good biological properties [2-6]. The silicate ones are known as mineral trioxide aggregates (MTA), presenting the same phase composition as Portland cement. MTA contains mainly calcium silicates ($3CaO{\cdot}SiO_2$ and $2CaO{\cdot}SiO_2$) and Bi_2O_3 (radio-opacifier), and, in small quantities tricalcium aluminate ($3CaO{\cdot}Al_2O_3$), calcium feritaluminate and calcium sulphate [1, 8-10].

On the other hand, bacterial cellulose or biocellulose with unique chemical and structural features has a lot of applications in biomedical fields such as biomaterial in tissue engineering, scaffolds, wound dressing, food packaging, drug release etc. [11-16].

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In this work, we have studied, for the first time, the influence of 10% or 33.34% biocelullose nanowiskers on the hardening processes of commercial MTA cement, the resulting materials being considered composite structures.

2. Materials and methods

We have used silicate cement, commercial MTA type, which has the following oxide composition: CaO, SiO_2, Bi_2O_3, Al_2O_3, Fe_2O_3, R_2O(R=Na, K), SO_3, MgO.

2.1.Production and purification of bacterial cellulose membranes

Bacterial cellulose or biocellulose (BC) membranes were produced by a strain belonging to Gram-negative, acetic acid bacteria *Gluconacetobacter* sp., isolated from traditionally fermented apple vinegar in Microbiology Laboratory of Chemical Engineering and Biochemical Department of University "Politehnica" of Bucharest. The culture was grown in a modified Hestrin-Schramm (MHS) medium [17], containing 2% fructose in a static culture. The pellicles obtained after predetermined periods of times (3, 5 and 7 days) were harvested and then were purified by treatment with 0.5 N NaOH aqueous solutions at 90 ºC for 1 h to eliminate the bacterial cells. The BC gel-like membranes were washed with deionized water until the water pH became neutral. BC pellicles were used as undried membranes.

2.2 Obtaining of biocellulose nanowhiskers

The hydrolysis of bacterial cellulose was carried out at room temperature for 4 days with continuous stirring, by adding 0.1 % bacterial cellulose into 100 ml H_2SO_4 aqueous solution (30% w/w). After 4 days, the hydrolysis was stopped by adding 400 ml deionized water. The pH was neutralized with NaOH solution (30% w/w). The prepared whiskers were centrifuged several times at 6000 rpm for 10 min. The obtained whiskers were dried from methanol and grinded for further use.

2.3. Composite preparation

The composite materials were obtained by mixing different amounts of MTA cement and biocellulose (Table 1). In order to investigate the influence of biocellulose on hardening processes of MTA cement, a witness sample was prepared from MTA cement, further referred as MTA-E.

Table 1. Composite materials composition

Composite symbol	MTA-E	MTA-10% biocell	MTA-33% biocell
MTA cement (% wt.)	100	90	66.66
Biocellulose (% wt.)	0	10	33.34

The powder/water ratio was 3:1. The slurry was prepared by strong mixing of the two components for 30 s at 23±2°C. The hardening was achieved at 37°C, 98% relative humidity, for 24 h.

Materials were investigated by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and thermal analysis (DTA). The composition and structure were examined by a Shimadzu XRD-6000 diffractometer. The composite morphology was analyzed with a Hitachi S-2600N and Quanta INSPECT F (equipped with electron field emission gun - EFG with a resolution of 1.2 nm) scanning electron microscopes. Biocellulose nanostructure was investigated with Tecnai G2 F30 S-TWIN transmission electron microscope. Complex thermal analysis was performed by using a Shimadzu DTG-TA-60 derivatograph.

2.4. Fluorescence microscopy

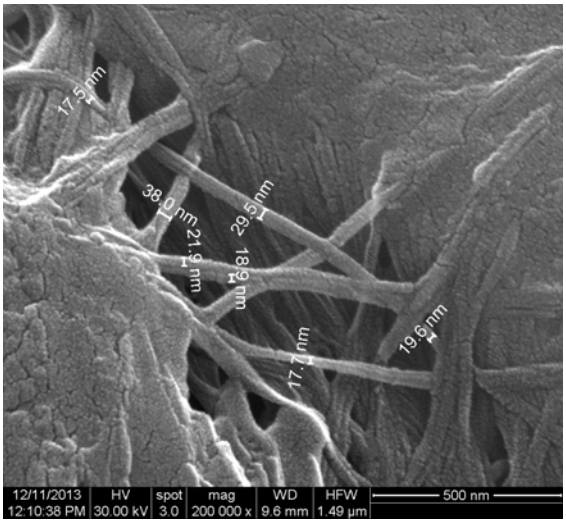
HEp (ATCC CLL 223^{TM}) cell line was cultivated in Dulbecco's Modified Essential Medium DMEM (Gibco, NY, SUA) supplemented with 10% heat-inactivated bovine serum and 1% penicillin/streptomycin at 37°C, with 5% CO_2, in a humid atmosphere.

After the treatment with 1 mg/ml MTA+33%biocel for 24h, HEp cells morphology and viability were quantified by double staining with 10µg/ml propidium iodide and 15 µg/mL fluorescein diacetate. The cell analysis by fluorescent microscopy was achieved using an Observer. D1 Carl Zeiss microscope and a AxioCamMR camera (Zeiss).

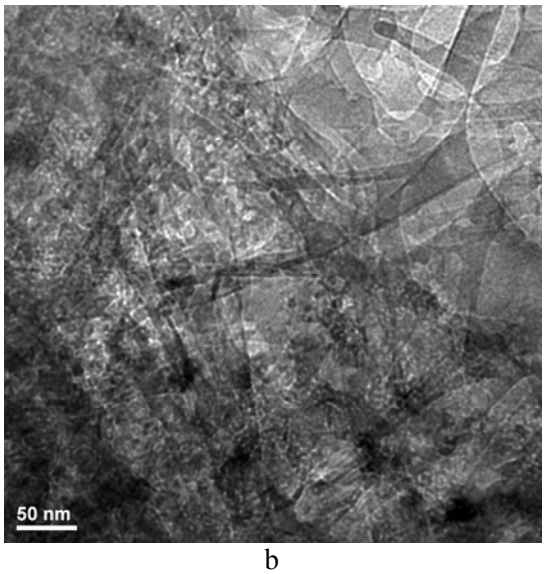
3. Results and discussions

3.1. Characterization of biocellulose nanowhiskers

The breadth of the resulting whiskers ranges between 15 and 40 nm (Fig. 1a), while theirs length is approximately 2-3 µm (fig.1.a). TEM image (Fig. 1b) indicate a good crystallinity, which means that the chemical processing did not affect the biocellulose original structure.



a



b

Fig. 1. (a) SEM and (b) TEM images of biocellulose nanowhiskers resulted after chemical processing of bacterial cellulose.

3.2. Composites characterization

Figure 2 displays the XRD patterns of anhydrous MTA cement and one day hardened MTA-E, MTA-10% biocell and MTA-33% biocell cements. It is obvious that after one day hardening, a decrease of the intensity of calcium silicates and calcium aluminate diffraction peaks appears. Moreover, it can be noticed the presence of new diffraction peaks in the case of one day hardened materials. Portlandit is the main compound resulted after hardening; some of it reacts with carbon dioxide from atmosphere, forming calcium carbonate. Furthermore, calcium hydrosilicates specific peaks appear in the XRD patterns of the studied compositions:

- 12.5 A, 3.07 A, 2.80 A and 1.83 A in the case of witness MTA cement, which is preponderant of type I (multifolded foils morphology) [18];
- 9.8 A, 3.07 A, 2.80 A and 1.83 A in the case of biocellulose containing compositions (MTA-10% biocell and MTA-33% biocell), which is preponderant of type II (fibers/needle morphology) [18];
- 9.67 A, 4.83 A, 3.16 A, 3.04 A, 2.79 A, 2.35 A and 1.66 A specific for tobermorit compound; these peaks are more pronounced in the case of composite cement-biocellulose.

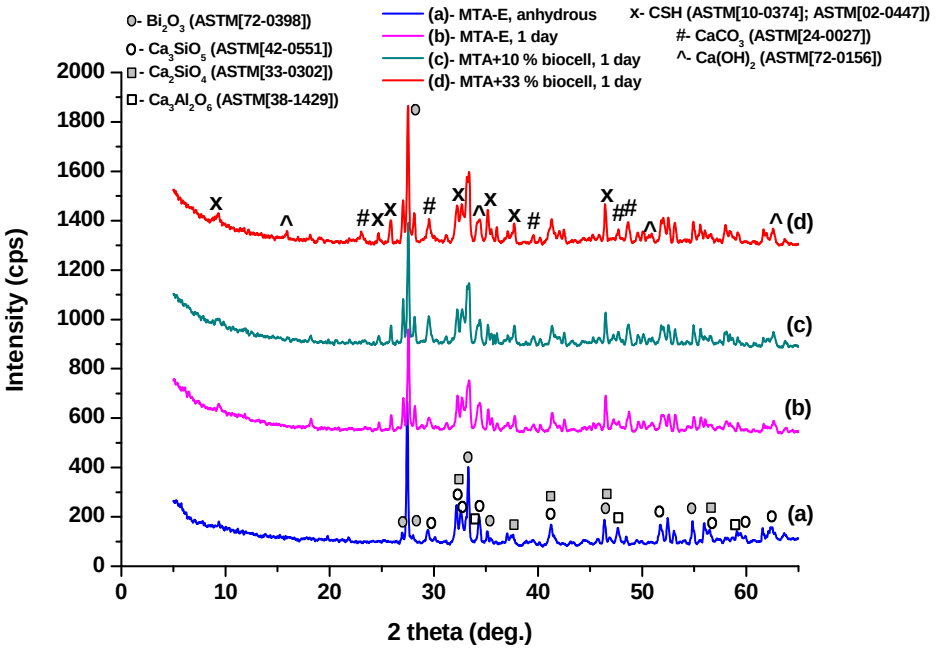


Fig. 2. XRD patterns of: (a) anhydrous MTA cement and one day hardened composites: (b) MTA-E, (c) MTA-10% biocell and (d) MTA-33% biocell.

The complex thermal analysis data for biocellulose and one day hardened composites are shown in Fig. 3 and 4 and gathered in Table 2.

Biocellulose burns approximately at 312°C, when an exothermic effect can be noticed, concomitantly with a strong weight loss. Moreover, other two endothermic effects appear at approximately 625°C and 711°C, which can be assigned to carbonates decomposition processes.

The curves obtained in the case of one day hardened composites indicate endothermic effects in the 90-200°C temperature range, attributed to semicrystalline hydrosilicates dehydration, also at approximately 450°C, related to portlandite dehydration and at approximately 680°C and 707°C, determined by the decomposition of low crystalline calcium carbonate. In the case of hardened composites, a exothermic effect accompanied by a weight loss appears (table 2), this being caused by biocellulose burning at the temperature around 320°C. Considering the thermal analysis data, it can be stated that the presence of biocellulose accelerates the hardening processes of MTA cement.

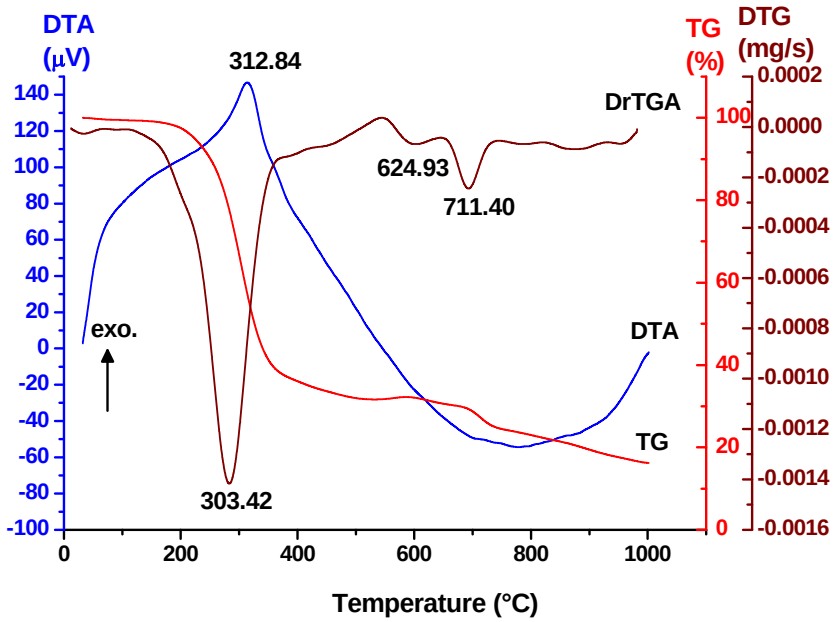


Fig. 3.Biocellulose thermal analysis.

Table 2. Weight loss values in the case of biocellulose and one day hardened composites.

Sample	Temperature range (C)					
	30-1000	30-283	283-385	385-500	500-1000	250-370
MTA-E, 1 day	14.50	6.68	0.82	1.67	5.33	0
MTA-10%biocell, 1day	19.53	7.76	2.47	1.29	8.01	3.13
MTA-33%biocell, 1 day	27.99	13.06	7.46	1.79	5.68	8.32
Biocellulose	83.79	22.29	40.71	4.98	15.81	52.10
		67.98				

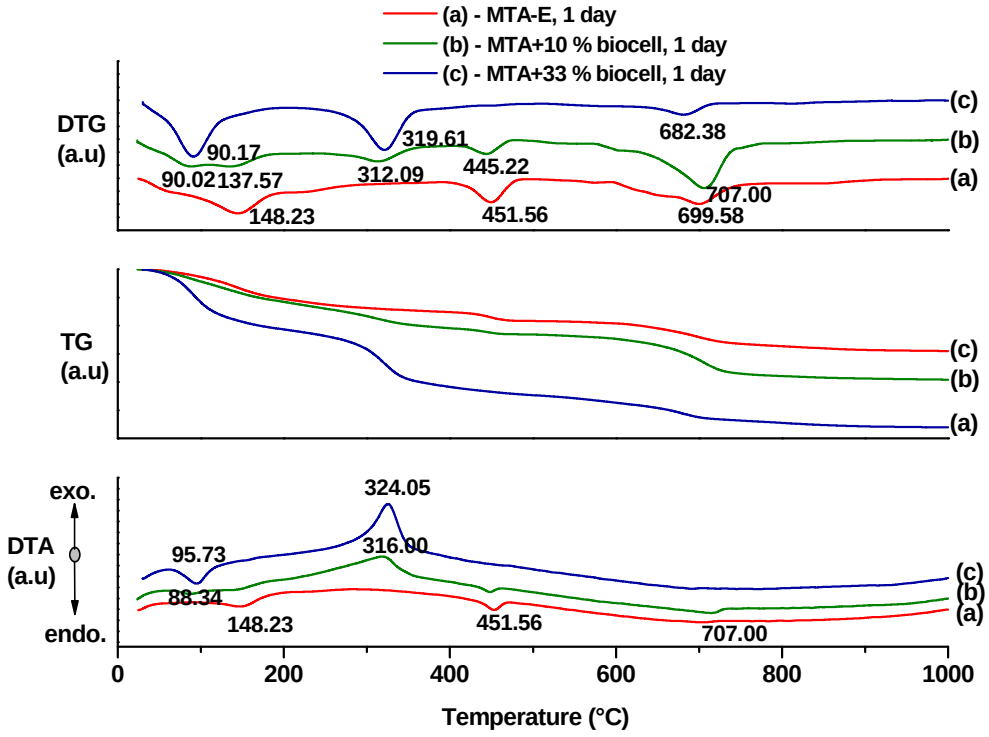


Fig. 4. Thermal analysis of hardened composites.

The morphology of one day hardened composites is presented in Fig. 5. In the case of the witness, the hydrosilicates appear in small quantities (fig. 5a, 5b), while the samples containing biocellulose nanowhiskers exhibit high amounts of acicular hydrosilicates, which can be associated with a coral shoal (fig 5c, 5d and fig 5e, 5f). These results are in good agreement with XRD patterns from figure 2. Peaks assigned to hydrosilicates corresponding MTA-33% biocell composite from figure 2d are stronger than that corresponding MTA-E identified as figure 2b. In the same time, in the case of MTA-33% biocell composite, (figure 2d) quantity of calcium hydroxide crystals decrease comparatively with figure 2b and 2c. Reaction equilibrium is modified and allows formation in a short period of time useful compounds for hardening structure. This type of images is reported for the first time in the literature and support the idea that biocellulose nanowhiskers favors the hydrosilicates formation.

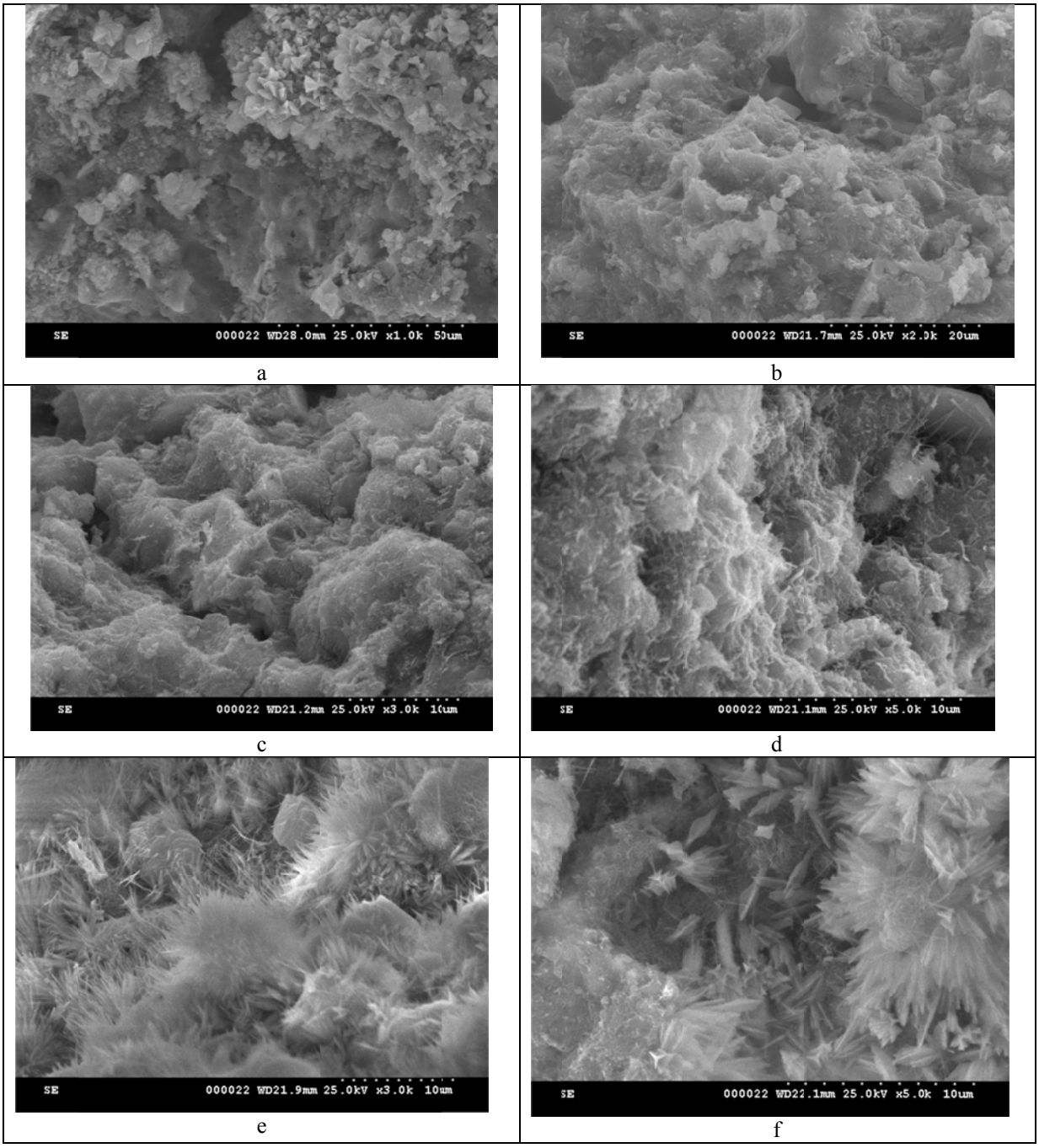


Fig. 5. SEM images of one day hardened composites: (a, b) MTA-E, (c, d) MTA-10% biocell and (e, f) MTA-33% biocell.

3.3. Composites biocompatibilty test

The cell morphology observed in both contrast phase and fluorescence fields microscopy (green micrographs) shows that MTA+33%biocel did not affect cell viability. HEp cells grown in the presence of 1 mg/ml MTA+33%biocel for 24h exhibit normal growth and morphology, their aspect and staining affinities being similar with the ones of the untreated control.

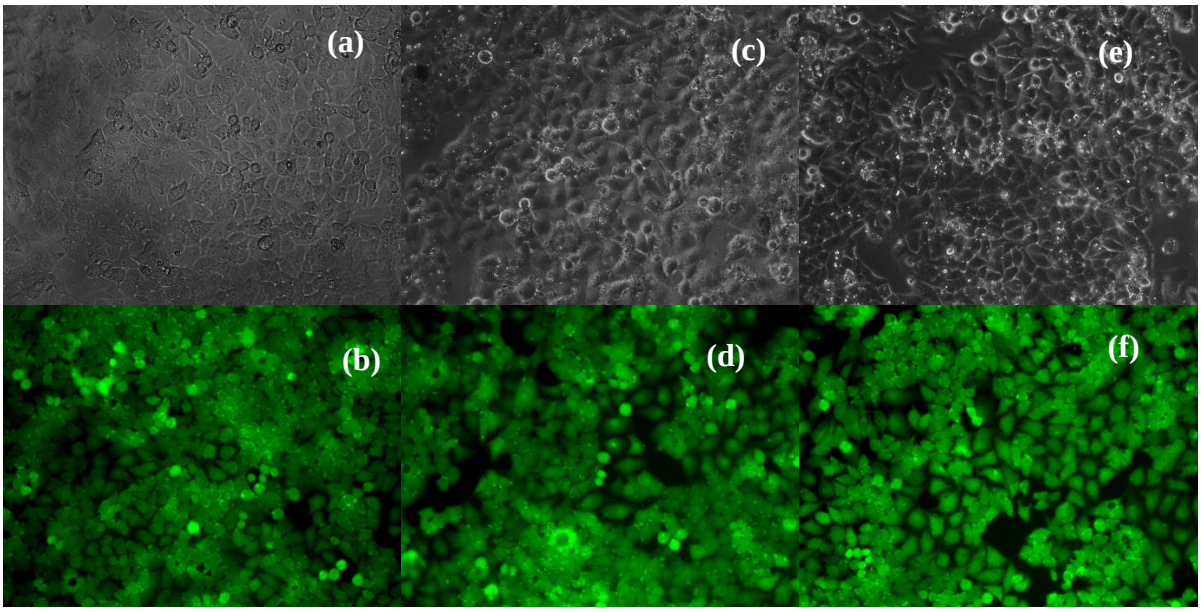


Fig. 6. The morphology of HEp cells treated with 1 mg/ml MTA+33%biocell: (a, c, e) contrast phase microscopy, (b, d, f) fluorescence microscopy, (a, b) HEp, 200x, (c, d, e, f) HEp cells treated with 1 mg/ml MTA+33%biocell, 200x.

4. Conclusions

A novel composite for dentistry use, made from MTA cement and biocellulose nanowhiskers has interesting features compared with commercial product. We reported for the first time in the literature that biocellulose nanowhiskers favor the hydrosilicates formation even after one day hardening. Biocompatibility test shows the same viability and affinity of cells as the untreated controlled samples. The obtained results encourage us to search new composite cement-biocellulose nanowhiskers which can be charged with nanoparticles of silica, calcium carbonate, magnetite, hidroxyapattite, silver, gold etc. Also, functionalized nanoparticles for drug release can be in view.

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