PREPARATION AND ANTIMICROBIAL ACTIVITY OF COLLAGEN/(RGO/ZnO/TiO_2/SiO_2) COMPOSITES

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ABSTRACT

A serial investigation is initiated aiming to explore the biological activity of some newly synthetized chemical compounds for the development of novel antimicrobial collagen based biomaterials. Collagen/$ZnTiO_3$, Collagen/RGO, Collagen/(Ag/RGO), Collagen/(Ag/RGO/SiO_2) and Collagen/($ZnTiO_3$/SiO_2) composites have been so far studied and all of them demonstrate a specific antimicrobial activity against Gram-negative and Gram-positive bacteria and in some cases against fungi. The aim of this investigation is to develop new antimicrobial collagen biomaterials using RGO, ZnO, and TiO_2 embedded in TEOS as another antimicrobial agent, combining the biological activity of RGO, ZnO and TiO_2 with the dispersing effect of SiO_2. The new Collagen/(RGO/ZnO/TiO_2/SiO_2) composites demonstrate an antimicrobial activity dependent on the agent loading level. It is specific in respect to Gram-negative, Gram-positive bacteria and fungi. An optimal balance between the antimicrobial activity and the cytotoxicity is achieved by varying the concentration of the antimicrobial agent, RGO/ZnO/TiO_2/SiO_2, in Collagen/(RGO/ZnO/TiO_2/SiO_2) composites. It is suggested that the mechanism of the antimicrobial action includes the simultaneous proceeding of (i) metal ions chelation; (ii) free oxygen radicals formation due to the interactions between the microbial cells and the antimicrobial agent; (iii) mechanical demolition of the cell walls and membranes by RGO crystal nanoparticles. The broad spectrum antibacterial and anti-fungal activity combined with the low cytotoxicity at an optimal Collagen/Antimicrobial agent ratio makes the studied Collagen/(RGO/ZnO/TiO_2/SiO_2) composites a promising antimicrobial material increasing the medical biomaterials assortment.

Keywords: antimicrobial agent; RGO/ZnO/TiO_2/SiO_2; Collagen/(RGO/ZnO/TiO_2/SiO_2) composites; antimicrobial activity; cytotoxicity.

INTRODUCTION

The increasing number of infections associated with the medical devices used and of the cases of microbial resistance observed in respect to antibiotics and multi-drug treatments applied, as well as the high prize of the healing and the negative issues for the patients require the development of new antimicrobial agents and biomaterials that would contribute to mitigation of the problem.

Collagen is a preferable biomaterial in medical coatings, tissue engineering scaffolds, membranes, wound dressings, etc. An antimicrobial activity is a desired property that stipulates the interest in the development of new collagen biomaterials, preferably of a wide spectrum of their antimicrobial activity. One easy and effective way, among a large variety of others, refers to the adding of an antimicrobial agent to collagen composites and coatings [1 - 4].

In several cases, metal nanoparticles or ions like those of silver, zinc, titanium and copper serve as an

antimicrobial agent affecting the bacterial life of the narrow-function bacterial taxa [5, 6].

Carbon materials like graphene, graphene oxide (GO) and reduced graphene oxide (RGO) have been lately studied as potential antimicrobial agents of a minimal toxicity in respect to mammalian cells. Their biocompatibility, high surface area, high mechanical strength, as well as the ability to induce a sustained stem cell growth and differentiations into various lineages are additional advantages [7-9]. Synergistic effects are reported for graphene-based nanocomposites containing metal and other antibacterial nanoparticles [10-12].

SiO_2 is accepted as one of the most promising carriers in the development of high performance antibacterial and bactericidal materials such as Ag-loaded SiO_2 (Ag/SiO_2) [13]. The ability of the silica matrix to improve the dispersion and hence to decrease the nanoparticles agglomeration is already well recognized [14 - 18].

This has led to the idea to develop antimicrobial collagen biomaterials utilizing newly synthetized nanocomposites. They are expected to deliver biologically active nanoparticles and ions as novel wide spectrum antimicrobial agents. The free $ZnTiO_3$ and that embedded in a silica matrix ($ZnTiO_3$/SiO_2), RGO, Ag-doped RGO and that embedded in TEOS, Ag/RGO/SiO_2 are among those used. It is found that Collagen/$ZnTiO_3$ [19], Collagen/($ZnTiO_3$/SiO_2) [17], Collagen/RGO [20], Collagen/(Ag/RGO) and Collagen/(Ag/RGO/SiO_2) [16] demonstrate a wide spectrum of a cell specific activity towards Gram-negative, Gram-positive bacteria, fungi and eukaryotes. The antimicrobial activity and the toxicity to eukaryotic cells depend on the loading level of the antimicrobial agent. Hence, the latter can be used for toxicity adjustment [16, 17, 19, 20].

No literature reports are found referring to the antimicrobial activity of ZnO and TiO_2 embedded in a silane matrix RGO, although the combined biological activity of RGO, ZnO and TiO_2 and the dispersing effect of SiO_2 promise a high antimicrobial activity of an wide spectrum. Therefore, the aim of the study reported refers to the utilization of the potential biological activity of RGO/ZnO/TiO_2/SiO_2 in the development of new Collagen/(RGO/ZnO/TiO_2/SiO_2) composites that would increase the assortment of collagen biomaterials of specific and optimal antimicrobial activity in case of different medical applications.

EXPERIMENTAL

Preparation of (RGO/ZnO/TiO_2 /SiO_2) composite

RGO, used in this investigation, was identical with that previously utilized [20]. ZnO nanoparticles (50 nm) were synthetized and characterized according to the procedures described in ref. [21]. TiO_2-nanoparticles (of an average size of 32 nm, a specific surface of 45 m^2/g and an anatas form) and tetraethoxysilane (TEOS, 98 %) were provided by Alfa Aesar. A simple hydrothermal method was employed to prepare the combined antimicrobial agent for this investigation, namely: RGO, ZnO and TiO_2 of 30 wt. %, 10 wt. % and 10 wt. %, respectively, were dispersed in distilled water. The mixture obtained was added to a ethanol solution of TEOS (1 : 1, v/v) under stirring for 1 h at 80°C. Few drops of HCl were added in order to obtain a gel. The latter was dried at 80°C for 2 h under vacuum to produce RGO/ZnO/TiO_2/SiO_2 powder.

Preparation of Collagen/(RGO/ZnO/TiO_2/SiO_2) composites

Type I fibril collagen gel of a concentration of 2.64 wt. % was extracted from calf hide using the previously described technology [22]. The concentration of the collagen gel was adjusted at 1 % and pH of 7.3 (that of the physiological medium) using 1M sodium hydroxide and (RGO/ZnO/TiO_2/SiO_2) powder was added to reach wt/wt ratios of 2 : 1, 2 : 0.8, 2 : 0.6, 2 : 0.4 or 2 : 0.2. The Collagen/(RGO/ZnO/TiO_2/SiO_2) nanocomposites obtained were cross-linked with 0.5 % glutaraldehyde (to dry collagen) at 4^0C for 24 h and then lyophilized at -40^0C to obtain a sponge material using a Martin Christ freeze-dryer for 48 h as previously described [23, 24].

SEM observations of the porous composites

JEOL SEM, model JSM-35 CF, Japan apparatus was used to observe the morphological features of the studied new antimicrobial agent (RGO/ZnO/TiO_2/SiO_2) and the new antimicrobial Collagen/(RGO/ZnO/TiO_2/SiO_2) composites. The samples were gold-sputtering coated and viewed in the second electron mode with a field emission gun.

A comprehensive modulus

A uniaxial compression testing was performed to evaluate the effect of RGO/ZnO/TiO_2/SiO_2 incorporated

in the collagen matrix on the comprehensive modulus of the corresponding composite. The mechanical testing machine Instron, TT-DM, USA was employed to carry out the testing at a strain rate of 10 % per minute. The results were averaged of 6 identical samples tested

An antimicrobial activity testing

Different types of microbial species: Gram-negative bacteria (*E. coli, Salmonela enterica*, and *Pseudomonas putida*, all of a small size cells*),* Gram-positive bacteria *(Stafilococus epidermidis* and *Bacilus cereus*, both of typical chains forming and large size cells) and fungus *Candida lusitaniae* (of a specific micelle organization) were used in this investigation.

All test microbial strains: *Salmonella enterica* 2333 (WDCM 00029, DSMZ 4224), *Pseudomonas putida* 1090 (ATCC 10536),*E. coli* 3548 (ATCC 10536), *Staphylococcus epidermidis* 3486 (ATCC 10536), *Bacilus cereus* 1095 (ATCC 11778) and *Candida Lucitaniae* (74-4) were provided by the Bulgarian National Bank of Microorganisms and Cell Cultures (NBIMCC), and cultured in the most suitable medium for them.

S. enterica 2333, *Escherichia coli 3548, Stafilococus epidermidis* 3486 were grown in a nutrient broth at 37°C and 180 rpm for 18 h. *B. cereus* 1095 and *Candida lusitaniae* 74-4 were propagated in a nutrient medium #14 NBIMCC (a beef extract, peptone, etc.) and YGC (VWR Prolabo Chemicals), respectively, at 30°C and 120 rpm. *Pseudomonas putida 1090 (ATCC 12633)* was cultivated in a synthetic liquid medium (ISO10712) at 22°C -23°C and 180 rpm for 12 h. The microbial density of 0.5 - 0,8 was determined according to McFarland. The aliquots of 100 μL microbial suspension were randomly spread on the solid medium (Nutrient agar – NA, #14 and YGS agar) and discs of the investigated material were put on them. The plates were left for 20 h at 4°C- 6°C to provide the nanoparticles diffusion and after that cultivated for 24 h at 37°C, 30°C and 24°C, respectively. The sterile zones formed around the disk samples (of a diameter of 9.0 mm and a thickness of 3 mm) were measured in mm (±0.5). The results were averaged on the ground of the measurement of at least 3 disk samples.

A cytotoxicity testing

In vitro cytotoxicity is tested following the requirements of ISO 10993–5 standard. A crystal violet assay is employed to quantify the viability of the three types of eukaryotic cells most often used in the tissue engineering: osteoblasts (MG-63), fibroblast (3T3) and kidney epithelial (MDCK II) cells, all provided from the NBIMCC, Bulgaria. The eukaryotic cells were maintained at standard conditions in a humidified atmosphere with 5% CO_2 at 37°C in F12 or DMEM (SIGMA) medium. The assessment of cytotoxicity was performed as described earlier [17].

RESULTS AND DISCUSSION

SEM images

Fig. 1 depicts the morphology of RGO/ZnO/TiO_2/SiO_2 used in this study as an antimicrobial agent. Aggregates of a layered structure and different dimensions are clearly seen. Most of them are larger than 1 μm.

Fig. 2 illustrates the porous structure of Collagen/(RGO/ZnO/TiO_2/SiO_2) composite at a collagen:an antimicrobial agent weight ratio of 2:1. The pictures of the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites of lower

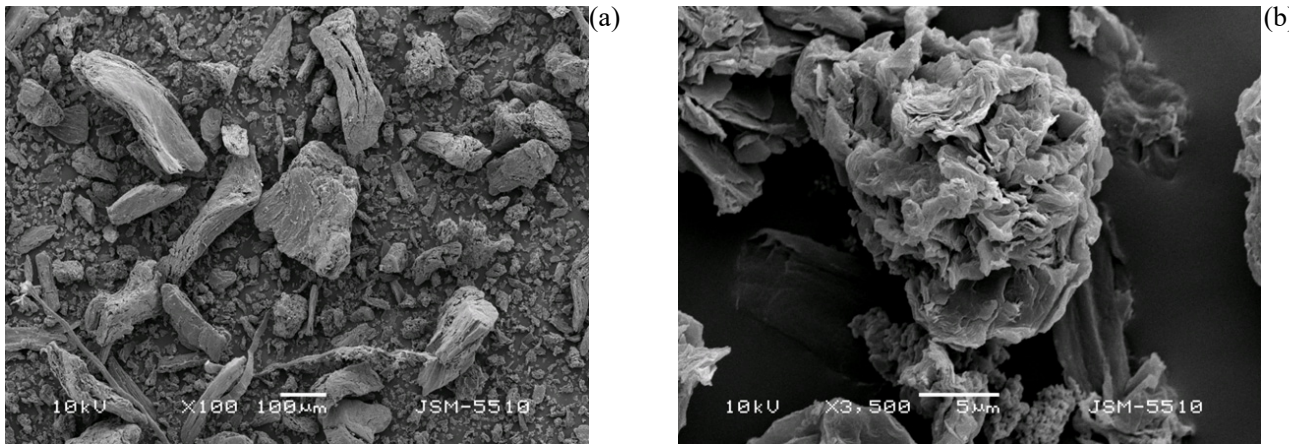
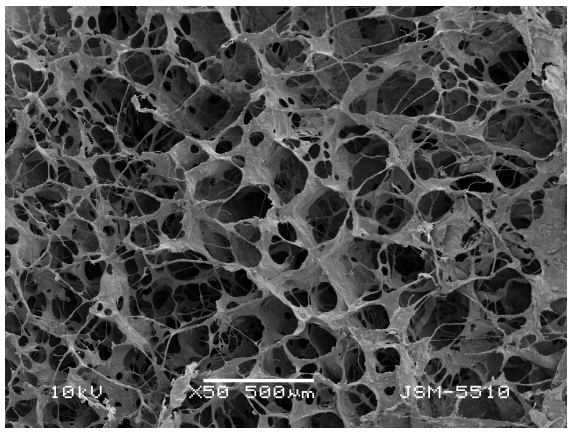
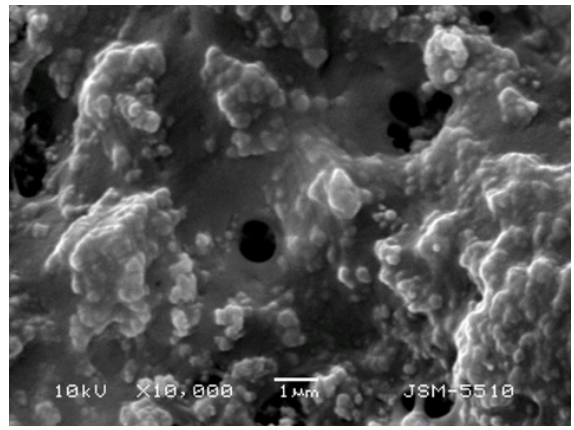


Fig. 1. SEM images of (RGO/ZnO/TiO_2/SiO_2) aggregates at different magnifications: (a) - x100; (b) - x3500.



(a)



(b)

Fig. 2. SEM images of Collagen/(RGO/ZnO/TiO_2/SiO_2) composite, wt : wt = 2 : 1, at different magnifications: (a) - x50; (b) - x10 000.

concentrations of the antimicrobial agent, namely at wt/wt ratios of 2:0.8; 2:0,6 and 2:0.4, are similar and which is why they are not presented here. Fig. 2 (a) depicts the open and interconnected relatively homogeneous porous structure of Collagen/(RGO/ZnO/TiO_2/SiO_2) composite whereas the picture at a higher magnification (Fig. 2 b) shows how (RGO/ZnO/TiO_2/SiO_2) particles are entrapped in the collagen matrix and are covered by collagen. It is also evident that all (RGO/ZnO/TiO_2/SiO_2 particles entrapped in the collagen matrix are of a submicron dimension, i.e. they are much smaller compared to RGO/ZnO/TiO_2/SiO_2 aggregates prior to the mixing with the collagen. This result confirms the fact that the presence of silica coating (TEOS) on the particles of the antimicrobial agent leads to improved dispersion and altered collagen-antimicrobial interface interaction as observed in case of Ag/RGO/SiO_2 [16] and $ZnTiO_3$/SiO_2 [17].

A comprehensive modulus

The compressive modulus of Collagen/(RGO/ZnO/TiO_2/SiO_2) composites at 10 % deformation is estimated expecting that the presence of RGO could influence their mechanical strength. The test results are presented in Table 1. The compressive modulus of two Collagen/RGO composites prepared under identical conditions (by sol-gel cryogen drying and the same collagen) [20] is presented to compare the reinforcing effect of (RGO/ZnO/TiO_2/SiO_2) with that of RGO. The Collagen/(RGO/ZnO/TiO_2/SiO_2) composites studied demonstrate an increased M_{10} modulus dependent on the (RGO/ZnO/TiO_2/SiO_2) concentration (Table 1, samples 2, 3, and 4) as compared to that of the collagen matrix (Table 1, sample 1). The same results have been obtained with the Collagen/RGO composites [20]. (RGO/ZnO/TiO_2/SiO_2) increases its mechanical strength keeping the interconnected porous structure (Fig. 2 (a)) of the corresponding collagen composite. This effect is better expressed at higher loading levels (compare samples 2, 3 and 4 in Table 1) at which the amount of RGO contained in the collagen composite is greater. A comparison of M_{10} modulus of samples 2 and 3 with that of samples 6 and 7 demonstrates the lower reinforcing effect of (RGO/ZnO/

Table 1. A compressive modulus M_{10} (KPa) at 10 % deformation of porous collagen, Collagen/(RGO/ZnO/TiO_2/SiO_2) and Collagen/RGO composites.

Sample	M_{10}, KPa
Collagen matrix	15. 6
Collagen/(RGO/ZnO/TiO_2/SiO_2) (2:1.0, wt/wt)	25.3
Collagen/(RGO/ZnO/TiO_2/SiO_2) (2:0.8, wt/wt)	24.1
Collagen/(RGO/ZnO/TiO_2/SiO_2) (2:0.6, wt/wt)	19.3
Collagen/(RGO/ZnO/TiO_2/SiO_2) (2:0.4, wt/wt)	18,6
Collagen/RGO (2:1.0, wt/wt) [20]	29.3
Collagen/RGO (2:0.8, wt/wt) [20]	26.6

TiO_2/SiO_2) compared to that of RGO. The increased values of M_{10} modulus of the samples containing RGO, compared with those of the samples containing the same amount of (RGO/ZnO/TiO_2/SiO_2) as well as the increased M_{10} modulus at higher (RGO/ZnO/TiO_2/SiO_2) loading levels (higher RGO levels, respectively) indicate that the reinforcing effect observed is mainly due to the presence of RGO. This result has already been verified by other authors [25].

An antimicrobial activity

The antimicrobial activity of the collagen composites loaded with (RGO/ZnO/TiO_2/SiO_2) observed as a sterile zone in mm is presented in Table 2 (samples 1-5). That of the collagen compounds loaded with the same amounts of $ZnTiO_3$ (Table 2, samples 1^a - 5^a) or RGO (samples 1^b - 5^b) is also presented for a comparison. All results in the table refer to the average value of 5 measurements with a deviation of ± 05 mm.

It is evident that the Collagen/(RGO/ZnO/TiO_2/SiO_2) porous collagen composites (Table 2, samples 1-5) demonstrate a concentration dependent specific antimicrobial activity against Gram-negative bacteria, *E. coli, S. enterica* and *P. putida* (Table 2, the first 3 column), Gram-positive bacteria, *Staphylococcus epidermidis* and *Bacillus cereus* and the fungus, *Candida lusitaniae* (Table 1, the last 4 columns). The specific activity could be due to the different shape, size, organization and structure of the test microbes. For example, *E. coli, Salmonela enterica*, and *Pseudomonas putida* have small size cells, *Stafilococus epidermidis* and *Bacilus cereus* have typical chains forming and large size cells, while the fungus *Candida lusitaniae* has a specific micelle organization. The well-expressed activity towards Gram-negative bacteria, Gram-positive bacteria and fungi, although at different loading levels for the different microbial strains, indicates the wide spectrum antimicrobial activity of the studied Collagen/(RGO/ZnO/TiO_2/SiO_2). It is greater than that of both Collagen/RGO (Table 2, samples 1^b - 5^b) and Collagen/$ZnTiO_3$ composites (Table 2, samples 1^a - 5^a). The Collagen/RGO composites demonstrate no activity against Gram-negative bacteria, they have a specific antifungal activity and some activity towards Gram-positive bacteria, like *B. cereus*. The Collagen/$ZnTiO_3$ composites demonstrate an activity towards Gram-positive bacteria and fungi but no activity at all loading levels in respect to *P. putida* and at low loadings levels to other Gram-negative bacteria like *S. enterica*

Table 2. An antimicrobial activity (as a sterile zone, mm) of collagen composites containing different amounts of the antimicrobial agent (AMA) : (RGO/ZnO/TiO_2/SiO_2), ($ZnTiO_3/SiO_2$)[a] and RGO[b].

Sample No	Collagen/AMA wt : wt	*E. coli*	*S. enterica*	*P. putida*	*S. epidermidis*	*B. cereus*	*C. lucitaniae*
	2:1						
1	Coll./(RGO/ZnO/TiO_2/SiO_2)	10.2	6.1	5.1	16.0	6.5	16.1
1^a	Collagen/$ZnTiO_3$	8.3	6.6	0	12.0	10.0	13.0
1^b	Collagen/RGO	0	0	0	0	16.1	17.9
	2:0.8						
2	Coll./(RGO/ZnO/TiO_2/SiO_2)	7.9	9.8	5.3	16.0	6.2	17.2
2^a	Collagen/$ZnTiO_3$	6.5	4.5	0	8.2	9.2	9.6
2^b	Collagen/RGO	0	0	0	0	2.0	10.5
	2:0.6						
3	Coll./(RGO/ZnO/TiO_2/SiO_2)	8.5	6.0	5.0	14.2	5.7	12.2
3^a	Collagen/$ZnTiO_3$	3.5	2.5	0	6.5	8.0	6.2
3^b	Collagen/RGO	0	0	0	0	1.9	6.5
	2:0.4						
4	Coll./(RGO/ZnO/TiO_2/SiO_2)	3.0	2.5	2.6	10.8	3.3	11.2
4^a	Collagen/$ZnTiO_3$	2.5	0	0	4.5	8.6	1,5
4^b	Collagen/RGO	0	0	0	0	0	5.3
	2:0.2						
5	Coll./(RGO/ZnO/TiO_2/SiO_2)	1.6	3.0	1.1	6.0	3.0	8.0
5^a	Collagen/$ZnTiO_3$	0	0	0	1.5	7.2	0.6
5^b	Collagen/RGO	0	0	0	0	0	1.2

and *E. coli*. The wide spectrum antimicrobial activity of the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites (Table 2, samples 1 - 5) is similar to that of Collagen/($ZnTiO_3$/SiO_2) composites found in a former investigation [17]. The antimicrobial agents, both (RGO/ZnO/TiO_2/SiO_2) and ($ZnTiO_3$/SiO_2), contain SiO_2 that outlines the importance of the dispersing effect of their embedding in the silicon matrix. The antimicrobial activity which is higher in value and enlarged in scope is attributed to the more homogenous distribution of the antimicrobial agent in a form of submicron (RGO/ZnO/TiO_2/SiO_2) aggregates in the collagen matrix as seen in Fig 2 (b). The specific activity towards different microbial cells could be ascribed to their size, shape, cell membrane and walls. The high, wide spectrum activity against bacteria and fungi makes the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites a promising antimicrobial biomaterial.

Cytotoxicity

A variety of the potential applications of the new antimicrobial Collagen/(RGO/ZnO/TiO_2/SiO_2) composites requires a low or lack of cytotoxicity. Three types of eukaryotic cells most often used in tissue engineering: osteoblast, MG-63, fibroblast, 3T3 and kidney epithelial, MDCK II are used to evaluate the cytotoxicity of the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites studied. The results are presented in Table 3. It is evident, that the viability of all test eukaryotic cells depends on the concentration of the antimicrobial agent, (RGO/ZnO/TiO_2/SiO_2), in the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites. It is relatively good. It is above 50% at all loading levels of the antimicrobial agent in case of the living fibroblasts, 3T3. The viability of the living kidney epithelial, MDCK II cells, is close to 50% at the highest concentrations (wt. ratios of 2 : 1 and 2 : 0.8) of the antimicrobial agent and higher than 50 % at lower concentrations (wt. ratios of 1:0.6; 2:0.4 and 2:0.2). The latter conditions ensure viability of the living osteoblasts, MG 63, also greater than 50 %. This gives a reason to accept that the newly developed antimicrobial Collagen/(RGO/ZnO/TiO_2/SiO_2) composites are relatively low toxic. An additional adjustment is provided by varying the loading level of the antimicrobial agent.

(RGO/ZnO/TiO_2/SiO_2) in a form of aggregates of a size greater than 1 μm is used in this study as an antimicrobial agent. It is entrapped in the collagen matrix by sol-gel cryogen drying to preserve the native biological activity of the collagen. No chemical interactions are expected between the collagen and (RGO/ZnO/TiO_2/SiO_2) under these conditions. Both, an easy destruction of (RGO/ZnO/TiO_2/SiO_2) aggregates and an easy engulf of the fine submicron particles obtained as a result of the destruction are observed during the mixing. This results in their relatively homogenies distribution in the collagen matrix (as evident in Fig. 2(b)) that contributes to the increased biological activity.

The antimicrobial agent, (RGO/ZnO/TiO_2/SiO_2), consists of RGO multilayered sheets as well as ZnO and TiO_2 anatas nanoparticles covered entirely by SiO_2. Unfortunately, the mechanism of the biological activity of such combined (RGO/ZnO/TiO_2/SiO_2) antimicrobial agent has not been so far studied. It is worth adding that the mechanism of the antimicrobial activity of RGO, ZnO and TiO_2 is still not fully understood [26-32].

The most frequently proposed mechanisms of graphene materials action include: (i) induction of oxidative

Table 3. A crystal violet assay of eukaryotic cells: osteoblast, MG-63, fibroblast, 3T3 and kidney epithelial, MDCK II cells on Collagen/(RGO/ZnO/TiO_2/SiO_2) composites.

Eukaryotic cells viability %	Collagen/(RGO/ZnO/TiO_2/SiO_2) composites, wt. : wt.				
	2 : 1	2 : 0.8	2 : 0.6	2 : 0.4	2 : 0.2
MG-63	39±3	42±16	59±13	72±6	89±12
3T3	63±9	79±1	85±2	92±16	96±2
MDCK II	46±12	49±10	76±2	86±5	98±2

stress with or without production of reactive oxygen species; (ii) a protein dysfunction; (iii) a membrane damage and (iv) a transcriptional arrest. The mechanism of action depends on the concentration of the graphene oxide (GO): the low GO concentrations provide the cutting of the membranes of *S. aureus* and *E. coli*, whereas the high concentrations induce the formation of GO aggregates shielding their edges. Bacterial deactivation through wrapping is observed [33 - 35] when the cluster size increases.

Synergistic effects are reported for graphene-based nanocomposites containing metal nanoparticles and ions. The chelation of the metal ions in Collagen/(RGO/ZnO/TiO_2/SiO_2) nanocomposite could also contribute to its biological activity. Such chelation could occur between the metal ions of the antimicrobial agent and the free electron couples of the oxygen and nitrogen atoms of the peptide bonds. The negative charge of the microbial cell wall observed under the test conditions could be a reason for the intake of the released metal ions chelated with collagen molecules. The metal ions cause toxicity to any cells [36 - 38] when engulfed at sufficiently high concentrations.

Based on the knowledge up to date, three mechanisms of action in respect to the antimicrobial activity of Collagen/(RGO/ZnO/TiO_2/SiO_2) composites could be assumed: (i) mechanical demolition of the cell wall and membrane by the antimicrobial agent nanoparticles entrapped in the collagen matrix; (ii) chelation of the deliberated metal ions in Collagen/(RGO/ZnO/TiO_2/SiO_2) composites and (iii) formation of reactive oxygen species due to the interactions between the antimicrobial agent and the microbial envelope.

CONCLUSIONS

New Collagen/(RGO/ZnO/TiO_2/SiO_2) composites of an well-expressed antimicrobial activity towards Gram-negative, Gram-positive bacteria and fungi combined with a relatively low cytotoxicity can be prepared by sol-gel cryogenic drying using (RGO/ZnO/TiO_2/SiO_2) as an antimicrobial agent.

Both, the antimicrobial activity in respect to different test microbial species and the cytotoxicity in case of different eukaryotic cells are specific. They depend on the loading level of the antimicrobial agent, (RGO/ZnO/TiO_2/SiO_2), presumably attributed to its cells specific size, shape, structure and other features.

An optimal balance between the antimicrobial activity and the cytotoxicity can be achieved by varying the loading level of (RGO/ZnO/TiO_2/SiO_2).

The wide spectrum antibacterial and antifungal activity combined with the low cytotoxicity determine the Collagen/(RGO/ZnO/TiO_2/SiO_2) composites as a promising antimicrobial biomaterial for medical applications like wound dressing, coatings, tissue engineering, etc.

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