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Original Article / Orijinal Araştırma

In vitro amoebicidal activity of poly(maleic anhydride-*co*-vinyl acetate) copolymer on *Acanthamoeba* spp. trophozoites and cysts

Acanthamoeba suşlarının trofozoit ve kistleri üzerine poli(maleik anhidrit-*ko*-vinil asetat) kopolimerinin in vitro amobisidal aktivitesi

Gülderen Karakuş[1,1], Erdoğan Malatyalı[2], HacıBayram Zengin[3], Serpil Değerli[2]

[1]Research Center of Cumhuriyet University School of Medicine, [2]Department of Parasitology, Faculty of Medicine, [3]Department of Chemistry, Cumhuriyet University, 58140 Sivas

Abstract

Aim. Poly(maleic anhydride-*co*-vinyl acetate) copolymer (MAVA) has been known to confer antitumor activity and it also exhibit many other biological activities. The amoebicidal effect of MAVA has not been investigated. **Methods.** In this study, for the first time amoebicidal effect of MAVA was investigated on *Acanthamoeba* spp. trophozoites and cysts. Trypan Blue Dye Exclusion test was used to determine the cell viability. **Results.** Our findings indicated that in the period of 48 h, the percentage and number of viable trophozoite showed a slow decrease except the dose of 32.0 mg/mL. The copolymer in the highest dose inhibited the proliferation of trophozoites in 3 h. The cysts were more resistant than the trophozoites to the inhibitory effect of copolymer. Structural characterization of the copolymer was confirmed by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (^{1}H-NMR). Surface morphology was visualized by both scanning electron and atomic force microscopy. **Conclusion.** In the lights of these data it could be suggested that MAVA deserves deeper investigation to improve its amoebicidal activity for both trophozoites and cysts forms of *Acanthamoeba* spp.

Keywords: poly(maleic anhydride-*co*-vinyl acetate) copolymer; *Acanthamoeba* spp. trophozoite; *Acanthamoeba* sp. cysts; amoebicidal activity; bioactive polymers.

Özet

Amaç. Poli(maleik anhidrit-*ko*-vinil asetat) kopolimerinin (MAVA) antitümoral aktiviteye sahip olduğu bilinmektedir ve aynı zamanda çeşitli biyolojik aktivitelere

[1] Corresponding Author:
Dr. Gülderen Karakuş, CÜTFAM, Cumhuriyet Üniversitesi Tıp Fakültesi, TR-58140 Sivas.
Email: gulderenkarakus@gmail.com

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sahiptir. MAVA'nın amobisidal etkisi daha önce incelenmemiştir. **Yöntem.** Bu araştırmada ilk kez MAVA'nın amobisidal aktivitesi *Acanthamoeba* trofozoit ve kistleri üzerinde incelendi. Hücre canlılığını belirlemek için Tripan mavisi boyası kullanıldı. **Bulgular.** Bulgularımız ilk 48 saatte 32,0 mg/mL dozu dışında canlı trofozoit oranı ve sayısının hızlı bir azalma gösterdiğini ortaya koydu. MAVA en yüksek dozunda 3 saat içinde trofozoitlerin çoğalmasını baskıladı. Kistlerin bu baskılayıcı etkiye daha dirençli oldukları saptandı. MAVA'nın yapısal karakterizasyonu Fourier Transform Infrared ve nükleer manyetik rezonans (^{1}H-NMR) ile doğrulandı. Yüzey morfolojisi ise hem taramalı elektron hem de atomik kuvvet mikroskobu ile görüntülendi. **Sonuç.** Bulgularımızın ışığında MAVA, *Acanthamoeba* trofozoit ve kistleri üzerinde gösterdiği amobisidal etkilerden dolayı daha fazla araştırılmayı hak etmektedir.

Anahtar sözcükler: Poli(maleik anhidrit-*ko*-vinil asetat) kopolimeri, *Acanthamoeba* spp. trofozoiti, *Acanthamoeba* spp. kisti, amobisidal aktivite, biyoaktif polimerler

Introduction

Maleic anhydride (MA) is a multifunctional chemical intermediates that find applications in nearly every field of industrial chemistry. It contains two acid carbonyl groups and a double bond in α, β position [1]. MA is a unique comonomer, because it does not readily undergo homopolymerization, but forms copolymers without difficulty. MA containing copolymers shows many and various biological activities; when they are incorporated into other monomers such as styrene, methyl methacrylate or vinyl acetate [2]. The first biological studies have been carried out for MA containing relatively simple polymer, maleic anhydride-divinyl ether (DIVEMA), using tumor cell lines [3]. Later on it has been shown that DIVEMA also possessed antiviral, antibacterial, and antifungal activities [4, 5].

Acanthamoebas are free-living microorganisms that can also act as opportunistic pathogens and are the causative agent of *Acanthamoeba* keratitis (AK) and Granulomatous Amebic Encephalitis (GAE) [6]. The infection of the human eye with parasite causes a sight-threatening keratitis [7]. The life cycle consists of two stages, a vegetative trophozoite stage and a dormant cyst stage. The trophozoite is motile, proliferates and feeds on other microorganisms such as bacteria, algae, yeasts or small organic particles. In adverse conditions, the trophozoites encyst and form a double wall containing cellulose. Noticeably, cysts are much more resistant to extreme environments than trophozoites [8]. *Acanthamoeba* sp. has spine-like structures on its surface called acanthopodia that distinguish it from other free-living amoeba.

The acanthopodia are crucially important in adhesion to surfaces, movement or capturing prey. *Acanthamoeba* can uptake of large volumes of solutes/particles by invagination of the plasma membrane and engulf large particles which may require specific interactions. Besides free-living in the soil, some species are opportunistic pathogen that can cause disease in humans and other animals. The other free-living amoebae that infect humans are *B. mandrillaris*, *N. fowleri*, and *Sappiniadiploidea*. The most common type of *Acanthamoeba* infection is *Acanthamoeba* keratitis (AK). Other infections include granulomatous amebic encephalitis (GAE) and full body infection. GAE is a very rare form of *Acanthamoeba* infection and characterized by a chronic progressive central nervous system infection. The infection usually affects immunocompromised individuals including AIDS patients and patients with a diabetes, malignancies, malnutrition, or

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systemic lupus erythematosus [9-11]. AK is the disease where amoebae invade the cornea of the eye and may lead to blindness. This type of disease is commonly associated with contact lens use, corneal trauma and history of exposure to possibly contaminated fresh water [12, 13].

The current AK treatment involves a combination of an amoebicidal drug, such as hexamidine (Desomedine®) or an aromatic diamidines (propamidineisethionate), with chlorhexidine gluconate or biguanides (PHMB) [14-16]. Isethionate is a water soluble compound consisting of a short chain alkane sulfonic acid containing hydroxy group. Chlorhexidine, a bisbiguanide antiseptic, is effective on bacteria, fungi and enveloped viruses. It is used at low concentrations in many products, such as mouthwash and contact lens solutions. Unlike AK, meningoencephalitis due to *Acanthamoeba* sp is thought to be unresponsive to chemotherapy and even today, there is no standardized treatment of this type of disease. However, an effective treatment of two cases is reported with an oral combination of ketoconazole, rifampicin and cotrimoxazole in an immunocompetent host [17].

So far the amoebicidal effect of MAVA or MA containing copolymer on Acanthamoebas has not been investigated [2, 18-20]. So this study involves amoebicidal effect of MAVA on *Acanthamoeba castellanii*, for both trophozoites and cysts forms. Our main goal is designing MA containing copolymers as a carrier for the drug. Furthermore antiamoebic drugs will be bound to MAVA backbone according to Ringsdorf model, proposing for polymer/drug conjugates. Further studies are needed on possible mechanisms and targets for amoebicidal activity of new polymer or polymer/drug conjugates.

Material and Methods

Materials

Maleic anhydride (MA), methyl ethyl ketone (MEK), and benzoyl peroxide (BPO) were obtained from Merck (Germany). Vinyl acetate (VA) and ethyl acetate were obtained from Sigma-Aldrich (USA). Ethyl alcohol and petroleum ether were obtained from Smyras (Teknik, Turkey).

Synthesis of MAVA copolymer

MAVA copolymer was synthesized by free radical polymerization of maleic anhydride (MA) and vinyl acetate (VA) at 1:1 molar ratio in methyl ethyl ketone (MEK) using benzoyl peroxide (BPO) as an initiator, at 80°C, for 24 h. Detailed information about this process can be found elsewhere [2, 21, 22]. Briefly, the polymerization of MA (recrystallized from benzene) and VA was carried out in a 100 mL three-necked round-bottom flask. MA (4.9 g, 50 mmol) was placed into the flask then dissolve in MEK (10 mL). To this solution VA (4.63 mL, 50 mmol) was added and stirred at room temperature for obtain homogeneos mixture. The copolymerization was started by adding BPO (0.05 g, 0.2 mmol) as a source of free radicals. Final volume of reaction was adjusted to 25 mL by adding MEK. The reaction media protected from light for homopolymerization reactions of VA. The resulting mixture was stirred continuously at 80°C for 24 h. Then it was purged continuously and allowed to continue until the first white precipitate products observed after the addition excess of cold ethyl alcohol (25 mL) at -20°C. The mixture was then cooled to room temperature. Unreacted vinyl acetate or its homopolymers were removed by dissolving the precipitate in ethyl acetate (20 mL) for 24 h and reprecipitating with

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petroleum ether. The final sample was filtered under vacuum and then dried at 55°C for 24 h.

Structural Characterization

MAVA copolymer was prepared as KBr pellets (2 mg sample in 100 mg KBr) and analyzed by Fourier Transform Infrared (FTIR) spectrophotometer (Mattson 1000, Unicam, USA) at 400 - 4000 cm^{-1} with 4 cm^{-1} increment. Nucleer Magnetic Resonance (^{1}H-NMR) analysis was performed at 400 MHz (BrukerAvance III, Karlsruhe, Germany) using 6 mg of the copolymer sample dissolved in 0.8 mL dimethyl sulfoxide (DMSO). Sample surface morphology was visualized by Scanning Electron Microscope (SEM) (LEO 440 Mensegi England). Sample surface morphology was also visualized by AFM (NanoScope VEECO, Manheim, Germany) from Digital Instruments operating in the tapping mode (not in contact with the sample) in air. MAVA sample was then suspended in ethyl alcohol and dried in droplets on silicon wafers. The images were taken at ambient temperature and humidity.

Preparation of Acanthamoeba castellanii *trophozoites and cysts*

Acanthamoeba castellanii cysts were kindly supplied from Dr. Beattie Tara (University of Strathclyde, Glasgow) on non-nutrient agar (NNA) plates. Prior to present study, axenisation was achieved by incubating them in 0.1 N HCl overnight in order to eliminate coexisting bacteria. Then, cysts were washed three times in distilled water. Finally, parasite incubated in axenic PPYG (protease peptone, yeast extract, glucose) medium and the trophozoites in the sixth day were further used in the assay [23]. The trophozoites were concentrated by centrifugation at 1500xg for 5 min [24]. The number of viable trophozoites was determined by trypan blue dye exclusion and direct trophozoite counts on a hemocytometer [25]. The final concentration was adjusted to $10x10^5$ trophozoites.mL^{-1} and the trophozoites were used in the assay immediately. Three week's old cultures of *Acanthamoeba castellanii* were used in cyst assay. The cysts were harvested by washing in distilled water and were adjusted a final concentration of $10x10^5$ cysts.mL^{-1} as previously described.

Assessment of the amoebicidal effect

MAVA was dissolved and diluted in distilled water. In fact, in aqueous solutions this copolymer is hydrolyzed to two neighbouring carboxyl groups in a structural unit and behaves as a dibasic polyacid [19]. Subsequently, *Acanthamoeba castellanii* trophozoites/cysts were exposed to serial concentrations (1, 2, 4, 8, 16 and 32 mg.mL^{-1}) of MAVA for 72 h. Trypan Blue Dye Exclusion test was used to determine the cell viability. The principle of the test is that live cells possess intact cell membranes that exclude the dye, whereas dead cells do not. For the test, 50 µL cell suspensions was simply mixed with the same volume of 0.5 % dye at certain hours; 1, 2, 3, 6, 8, 24, 48 and 72. The mixture was allowed to incubate 3 min at room temperature and then the unstained (viable) and stained (nonviable) cells were counted separately in the hemocytometer [26]. The experiments were repeated three times. The cell viability calculated as follows:

Viability % = The number of viable trophozoites / The total number of trophozoites (viable + nonviable)

For cultures containing no viable cysts, an additional test was performed to confirm the results obtained. In order to evaluate their viability, cysts were inoculated onto a non-

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nutrient agar plates covered with a lawn of Escherichia coli after the each incubation period. The culture plates were incubated at 30°C for two days [27].

Statistical analysis

Nonparametric tests were used in statistical analysis, because of the samples size (less than 30). The data were presented as mean values with standard deviations and analyzed by Kruskal-Wallis followed by Mann-Whitney U test for post-hoc pairwise comparisons. The Kruskal-Wallis test compares three or more unpaired groups. The Mann-Whitney U test is used in place of an unpaired t-test. The P-value was set at 0.05 for significance level.

Results and Discussion

FTIR Analysis

MAVA copolymer (Fig. 1) had anhydride units at 1880 cm^{-1}, and 1804 cm^{-1} as expected, indicating symmetric and asymmetric C=O stretching vibrations, respectively [28-30]. Characteristic C-O stretching vibrations at 1242 cm^{-1} and 1012 cm^{-1} and a CH_3 group of VA stretching vibration at 1395 cm^{-1} were observed [31]. These findings supported the MAVA copolymer structure as expected.

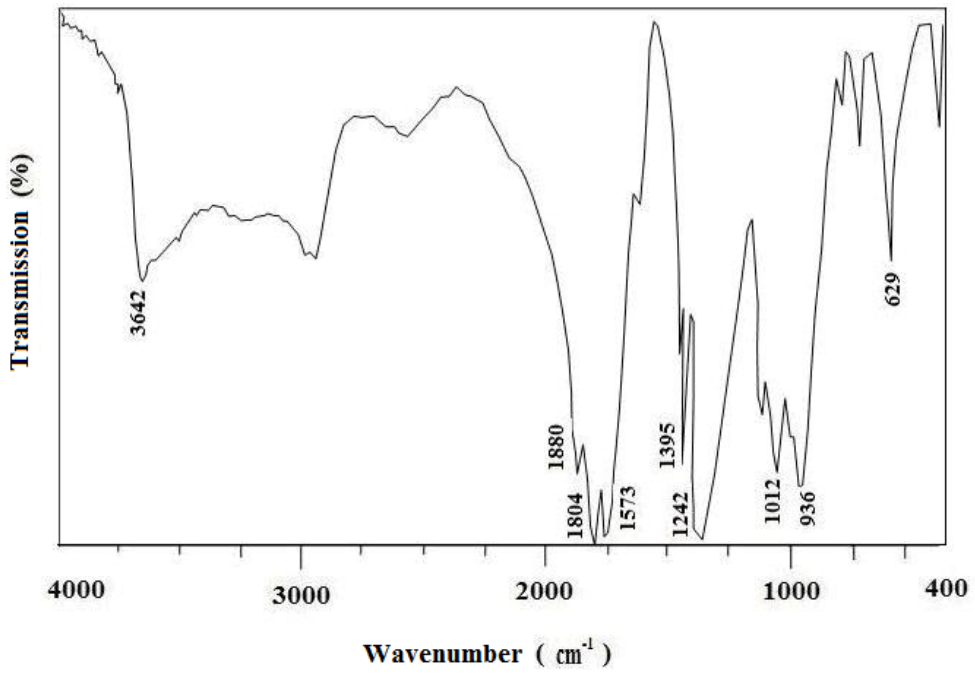

Figure 1. FTIR spectra of the MAVA copolymer.

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NMR Analysis

Characteristic features of the MAVA spectrum were a chemical shift of two protons on MA groups at 5.4 ppm, three methyl protons at 2 ppm [18, 19]. -CH2 protons on VA, approximately at 2 ppm; and a multiplet peak for –CH, bound to oxygen at 1.1 ppm (Fig. 2) [2, 30]. These findings supported the MAVA copolymer structure as expected.

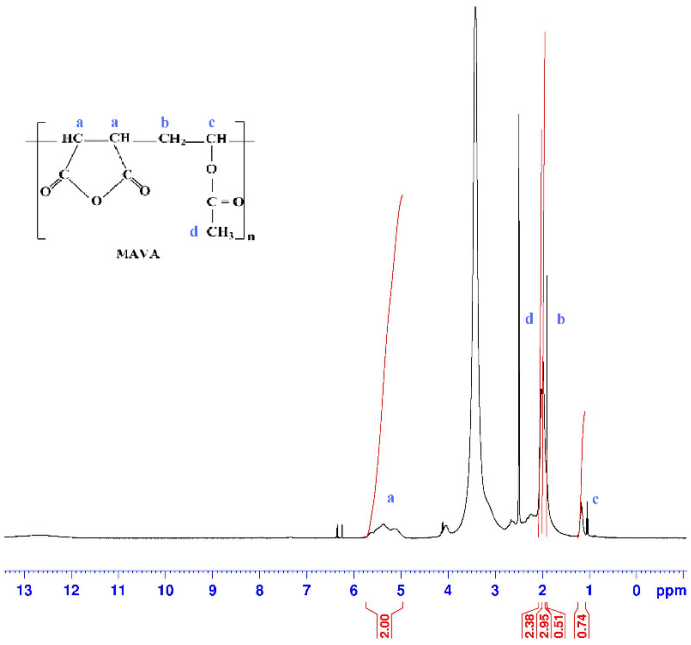

Figure 2. NMR spectra of the MAVA copolymer.

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SEM Images of the MAVA

Surface features of the copolymer were visualized by SEM at differing magnifications (MAVA at 20.00 KX-1 µm, 20.00 KX-2 µm, 10.00 KX-2 µm, and 5.00 KX-10 µm; KX, magnification; µm, resolution Fig. 3). In the images it was obvious that MAVA copolymer had surfaces characterized by evenly uniformly distributed cavities.

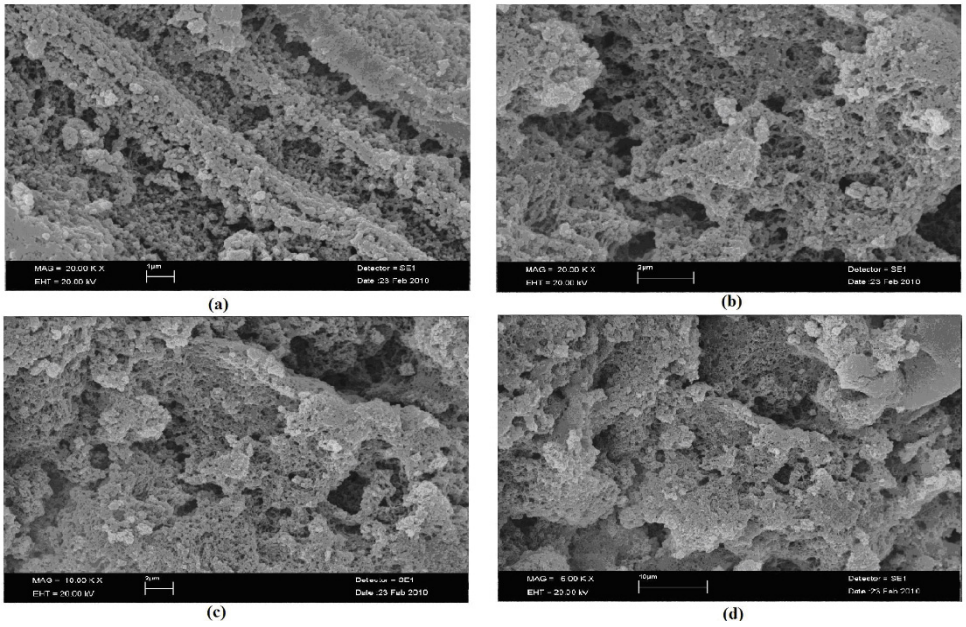

Figure 3. SEM images of MAVA copolymer: (a) 20.00 KX-1 µm, (b) 20.00 KX-2 µm, (c) 10.00 KX-2 µm, (d) 5.00 KX-10 µm.

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AFM Images of the MAVA

Surface morphology of the copolymer were also visualized by AFM, set at the tapping mode that has several advantages over the scanning electron microscope (SEM): AFM produces three-dimensional high resolution surface images, and samples do not require any prior treatments such as metal/carbon coatings that would irreversibly change or damage the sample [32, 33]. Surface features of MAVA were visualized by AFM at differing scan size and data scale values; 20.00 µm, 10.00 µm, 5 µm, and 2 µm; (Fig. 4a, 4b, 4c and 4d, respectively) [34]. Digital Instruments NanoScope values essentially demonstrated samples under the conditions their images were taken. MAVA copolymer was scanned at 1.001 Hz and nm or µm expressions indicated the area, of which the images were produced. By inspection of the images with naked eye it could easily be noted that the roughness of the MAVA was quite less and properly surface.

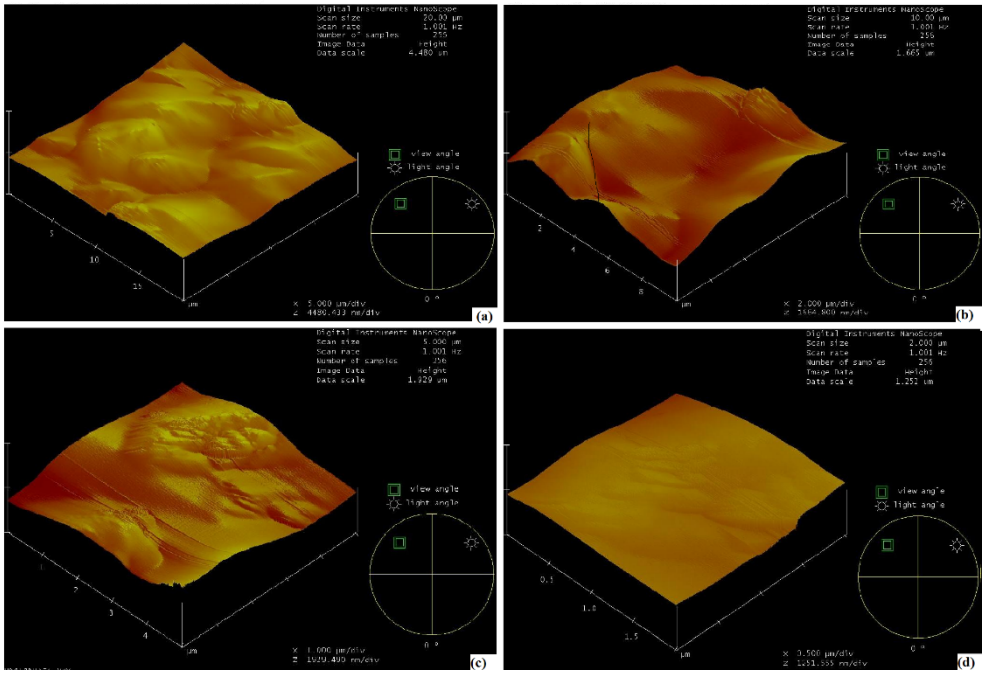

Figure 4. AFM images of MAVA copolymer: (a) 20 µm, (b) 10 µm, (c) 5 µm, (d) 2 µm.

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Amoebicidal effects of MAVA

The result of the *in vitro* effect of MAVA copolymer on *Acanthamoeba castellanii* trophozoites and cysts from one hour to 96 hours of incubation was summarized in Tables 1 and 2. MAVA had a dose and time dependent effect on *Acanthamoeba castellanii* with concentrations ranging between 1.0 and 32.0 mg.mL^{-1} (Figs. 5-7). The total number of viable trophozoites/cysts was different in all five different concentrations of MAVA as indicated by the Kruskal-Wallis test (trophozoites; K-W statistics =41.174, DF = 6, $P < 0.05$, cysts; K-W statistics = 31.419, DF = 6, $P < 0.05$).

Table 1. In vitro antiamoebic activity of polymer, against *Acanthamoeba castellanii* trophozoites.

MAVA (mg/mL)	Measurement times (h)						
	1	2	3	6	8	24	48
1	99.3±1.2	97.7±0.6	94.3±1.5	93.7±1.5	90.3±1.5	86.7±4.2	84.3±4
2*	97.3±1.2	95.3±0.6	92.3±1.5	89.3±4	86.7±4.5	85±5	82±4
4*	82.7±2.1	76.7±1.5	74.3±2.1	64.7±2.5	56.7±3.1	43.3±3.5	35.3±2.5
8*	77.7±3.8	72.7±3.5	60.3±3.8	47.3±3.1	39±1	22±2.6	0
16*	57.3±4.5	39.7±2.5	31.3±4.5	19.3±1.5	10.3±1.5	0	0
32*	21.0±3	12±3.6	0	0	0	0	0
Control	96.7±1.5	96.3±1.5	96±1	95.7±2.3	95.7±2.1	95.3±1.5	95

Data was presented as mean ± SD. *Significant differences from controls determined with Mann-Whitney test at $p<0.05$.

Table 2. In vitro antiamoebic activity of polymer, against *Acanthamoeba castellanii* cysts.

MAVA (mg/mL)	Measurement times (h)						
	1	2	3	6	8	24	48
1*	97±1	94.3±2.1	93±1	88±2	84.3±0.6	82±3	77±2.6
2*	94±1	92.3±1.5	88±2.6	82.3±1.5	80.3±1.5	74.7±1.5	73±1.7
4*	91±1.7	85.0±1	83.3±2.9	79.3±3.1	74.7±1.5	66±3	61.3±2.9
8*	87.7±1.5	82.0±3	75±3	67.7±2.5	62.3±2.5	52.7±2.5	38.7±1.5
16*	84.7±1.5	81.3±2.3	70.3±4.7	61±6.2	45.3±0.6	34.3±2.1	0
32*	83±4.6	71.3±1.2	59±2.6	47.3±3.2	33±3.5	0	0
Control	99.3±1.2	98.7±0.6	97±1	97±1.7	97±1	96.3±1.5	96±1.7

Values presented as mean ± SD. *Significant differences from controls determined with Mann-Whitney test at $p<0.05$.

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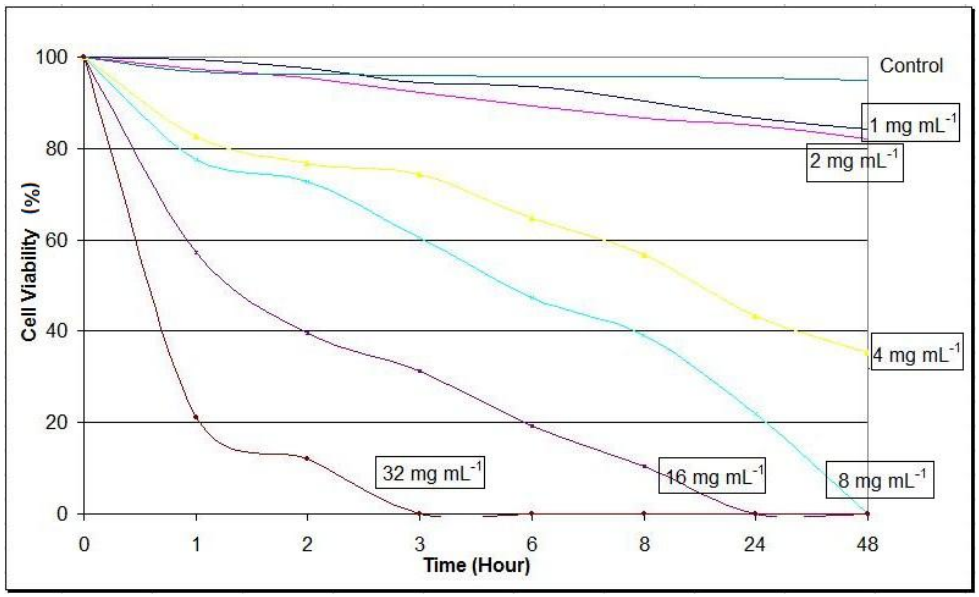

Figure 5. Effect of MAVA on the viability of *Acanthamoeba castellanii* trophozoites.

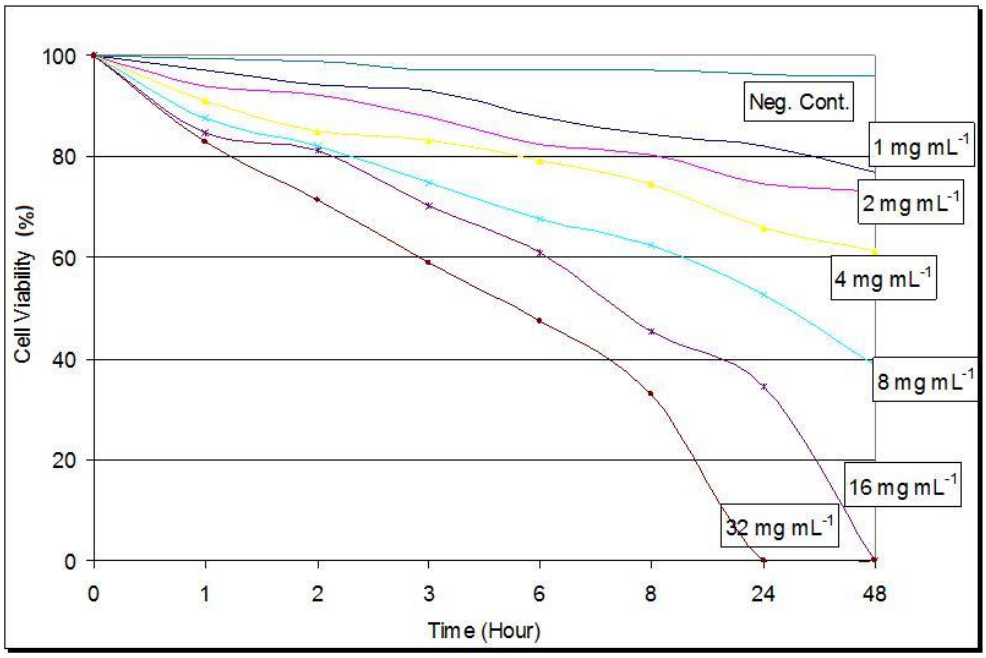

Figure 6. Effect of MAVA on the viability of *Acanthamoeba castellanii* cysts.

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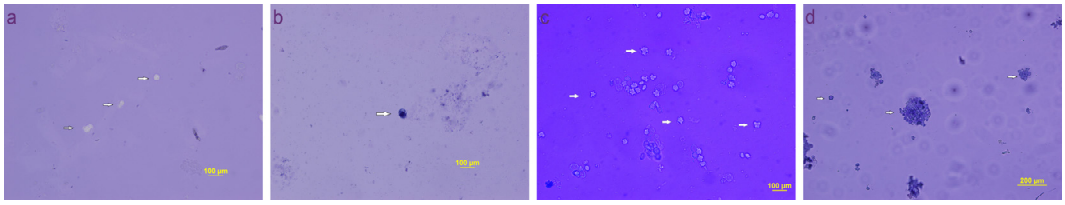

Figure 7. Viable and nonviable *A. castellani* trophozoites (a and b). (a) Three viable trophozoites of *A. castellani* before MAVA addition. (b) Stained (nonviable) trophozoite after three hours of incubation with MAVA (32 mg.mL^{-1}). Viable and nonviable *A. castellani* cysts (c and d). (c) Viable cysts of *A. castellani* before MAVA addition. (d) Stained (nonviable) cysts after 24 hours of incubation with MAVA (32 mg.mL^{-1}).

In the period of 48 h, the percentage and the number of viable trophozoites showed a slow decrease except the dose of 32.0 mg mL^{-1}. The copolymer in the highest dose inhibited the proliferation of trophozoites in three hours. The cysts were more resistant to the inhibitory effect of copolymer.

Chlorhexidine has amoebicidal activity, the mechanism of action being membrane disruption [35, 36]. It was expected that MAVA would not have an effect in such way, since the cells had been kept their anatomical shape even after treatment. Pinocytosis and/or phagocytosis are used as a means of feeding, providing part or all of their nourishment for Acanthamoebas. A fundamental step in phagocytosis is the receptor-mediated recognition of a prey such as mannose receptor [37]. It can be concluded that any substances that cover the cell surface may cause an inhibition in feeding of parasite. MAVA may have such an effect the kept shape of parasite may be a possible outcome. However, it has to be considered that the entrance of vital dye inside the cell will not affected from MAVA. It is too large to enter cell membranes hence it must be broken into small pieces. Many amoebicidal drugs are effective over cell membranes, a similar way of action could be suggested [38].

Many natural and synthetic products have been investigated for their antiamoebic activity and some plant products have been shown to inhibit the growth of parasite [10, 39-45]. The reported MIC doses in these studies ranged from 10 to 8 mg mL^{-1}. In the present study, we have demonstrated that higher level of MAVA inhibits cell proliferation and induces cell mortality of *Acanthamoeba* trophozoites. Further studies are needed on possible mechanisms and targets for amoebicidal activity of newer polymer.

Conclusions

So far the amoebicidal effect of MAVA or MA containing copolymer on Acanthamoebas has not been investigated. This study involves amoebicidal effect of MAVA on *Acanthamoeba castellanii*, for both trophozoites and cysts forms. We have demonstrated that higher level of MAVA inhibits cell proliferation and induces cell mortality of *Acanthamoeba castellanii* trophozoites. Further studies are needed on possible mechanisms and targets for amoebicidal activity of newer polymer. Furthermore antiamoebic drugs will be bound to MAVA backbone according to Ringsdorf model, proposing for polymer/drug conjugates. In the lights of these results it could be suggested

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that MAVA copolymer deserves further investigation in order to assess its amoebicidal effects on *Acanthamoeba* trophozoites and cysts.

Conflict of Interest

The authors of the manuscript solemnly declare that no scientific and/or financial conflicts of interest, exists with other people or institutions.

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References

1. Felthouse TR, Burnett JC, Horrell B, Mummey MJ, Kuo YJ. Chemical Technology 2001, DOI: 10.1002/0471238961.1301120506051220, Huntsman Petrochemical Corporation Austin Laboratories 7114 North Lamar Boulevard Austin, Texas, pp 1-58.
2. Spridon D, Panaitescu L, Ursu D, and Uglea CV. Synthesis and biocompatibility of maleic anhydride copolymers: 1. maleic anhydride-vinyl acetate, maleic anhydride methyl methacrylate and maleic anhydride-styrene. Polym Int. 1997;43:175-81.
3. Breslow DS. Biologically active synthetic polymers. Pure &Appl Chem. 1976; 46: 103-13.
4. Dhal PK, Holmes-Farley SR, Huval CC, and Jozefiak TH. Polymers as drugs. AdvPolym Sci. 2006;192:9-58.
5. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov. 2003 May;2(5):347-60.
6. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amabas. Brain Pathol. 1997 Jan;7(1):583-598.
7. Değerli S, Saygı G. Acanthamoebakeratiti. TurkiyeParazitolDerg. 2000;24:243-8.
8. Claerhout I, Kestelyn PH. Acanthamoeba keratitis: a review. Bull SocOphtalmol. 1999;274,71-82.
9. Walia R, Montoya JG, Visvesvera GS, Booton GC, Doyle RL. A case of successful treatment of cutaneous Acanthamoeba infection in a lung transplant recipient. Transpl Infect Dis. 2007 Mar;9(1), 51-4.
10. MacLean RC, Hafez N, Tripathi S, Childress CG, Ghatak NR, Marciano-Cabral F. Identification of Acanthamoeba sp. İn parafin-embedded CNS tissue from an HIV+ individual by PCR. DiagnMicrobiol Infect Dis. 2007 Mar; 57(3):289-94.
11. Rivera M, Padhya T. Acanthamoeba: a rare primary cause of chinosinusitis. Laryngoscope. 2002 Jul;112(7 Pt 1):1201-3.
12. Cabral FM, Cabral G. Acanthamoeba spp. As agents of disease in humans. ClinMicrobiol Rev. 2003 Apr;16(2), 273-307.
13. Pasricha G, Sharma S, Garg P, Aggarwal RK. Use of 18S rRNA gene-based PCR assay for diagnosis of Acanthamoeba keratitis in non-contact lens wearers in India. J

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ClinMicrobiol. 2003 Jul; 41(7):3206-11.

14. Sun X, Zhang Y, Li R, Wang Z, Luo S, Gao M, Deng S, Chen W, Jin X. Acanthamoeba keratitis: clinical characteristics and management. Ophthalmology, 2006 Mar;113(3), 412–6.
15. Dart JK, Saw VP, Kilvington S. Acanthamoeba keratitis: diagnosis and treatment update 2009. Am J Ophthalmol. 2009 Oct;148(4):487-499.
16. Ertabaklar H, Dayanir V, Apaydin P, Ertug S, Walochnik J. Case report: Acanthamoeba keratitis. TurkiyeParazitolDerg. 2009;33(4):283-5.
17. Singhal T, Bajpai A, Kalra V, Kabra SK, Samantaray JK, Satpathy G, Gupta AK. Pediatr Infect Dis J. 2001 Jun; 20(6), 623–7.
18. Yoon KJ, Woo JH, Seo YS. Formaldehyde free cross-linking agents based on maleic anhydride copolymers. Fiber Polym. 2003;4:182-187.
19. Nemtoi G, Beldie C, Tircolea C, Popa I, Cretescu I, Humelnicu I, Humelnicu D. Behaviour of the poly(maleic anhydride-co-vinyl acetate) copolymer in aqueous solutions. EurPolym J. 2001;37:729-35.
20. Xing CM, Yang WT. A novel, facile method for the preparation of uniform, reactive maleic anhydride/vinyl acetate copolymer micro- and nanospheres. Macromol Rapid Comm. 2004;25:1568-74.
21. Nguyen V, Yoshida W, Cohen Y. Graft polymerization of vinyl acetate onto silica. J ApplPolym Sci. 2003;87:300-310.
22. Karakus G, Zengin HB, Polat ZA, Yenidunya AF, Aydin S. Cytotoxicity of three maleic anhydride copolymers and common solvents used for polymer solvation. Polym Bull. 2012; DOI: 10.1007/s00289-012-0860-5.
23. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. ClinMicrobiol Rev. 2002 Jul; 15(3):342-54.
24. Garcia LS, Brucker DA, Diagnostic medical parasitology, 2nd Ed., American Society for Microbiology, Washington, pp 601 (1993).
25. Khan NA.Acanthamoeba Biology and Pathogenesis, Caister Academic Press, Norfolk, UK, pp 85 (2009).
26. Ballarin C, Peruffo A. Primary cultures of astrocytes from fetal bovine brain. Methods in Molecular Biology. 2012; 814:117-26.
27. Rendules O, Ferrières L, Frètaud M, Bègaud E, Herbomel P, Levraud JP, Ghigo JM. A new zebrafish model of oro-intestinal pathogen colonization reveals a key role for adhesion in protection by probiotic bacteria. Plos Pathogens. 2012;8(7);1-17.
28. Kaplan Can H, Doğan AL, Rzaev ZMO, Uner, AH, Güner A. Synthesis and antitumor activity of poly(3,4-dihydro-2H-pyran-co-maleic anhydride-co-vinyl acetate). J ApplPolym Sci. 2005;96:2352-59.
29. Pal J, Singh H, Ghosh AK. Modification of LLDPE using esterified styrene maleic anhydride copolymer: Study of its properties and environmental degradability. J ApplPolym Sci. 2004;92:102-8.
30. Popa I, Offenberg H, Beldie C, Uglea CV. Benzocaine modified maleic anhydride copolymers-I. Synthesis and characterization of benzocaine modified poly(maleic anhydride-co-vinyl acetate), poly(maleic anhydride-co-methyl methacrylate) and poly(maleic anhydride-co-styrene). EurPolym J. 1997;33:1511-14.
31. Qiao Z, Xie Y, Chen M, Xu J, Zhu Y, Qian Y. Synthesis of lead sulfide/(polyvinyl

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acetate) nanocomposites with controllable morphology. ChemPhysLett. 2000;321:504-7.

32. Giessibl F. Advances in Atomic Force Microscopy. J Rev Mod Phys. 2003;75: 949-83.
33. Cowman MK, Li M, Balazs EA. Tapping mode atomic microscopy of hyaluronan: extended and intramolecularly interacting chains. Biophys J. 1998 Oct;75(4), 2030-7.
34. Hernández JCR, Sánchez MS, Ribelles JLG, Pradas MM. Polymer-silica nanocomposites prepared by sol-gel technique: Nanoindentation and tapping mode AFM. EurPolym J. 2007;43:2775-2783.
35. Kuyyakanond T, Quesnel LB. The mechanism of action of chlohexidine. FEMS MicrobiolLett. 1992 Dec 15;79(1-3):211–5.
36. El-Sayed NM, Ismail KA, Ahmed SA, Hetta MH. In vitro amoebicidal activity of ethanol extracts of Arachis hypogaea L., Curcuma longa L. and Pancratium maritimum L. on Acanthamoeba castellanii cysts. Parasitol Res. 2012 May; 110(5):1985-92.
37. Allen PG, Dawidowicz EA. Phagocytosis in Acanthamoeba: I. A mannose receptor is responsible fort he binding and phagocytosis of yeast. J Cell Physiol. 1990 Dec; 145(3):508-13.
38. Roberts CW, Henriquez FL. Drug target identification, validation, characterisation and exploitation for treatment of Acanthamoeba (species) infections. ExpParasitol. 2010 Sep; 126(1):91–6.
39. Cariello AJ, Souza GFP, Foronda AS, Yu MCZ, Hofling-Lima AL, Ganzarolli M. In vitro amoebicidal activity of S-nitrosoglutathione and S-nitroso-N-acetylcysteine against trophozoites of Acanthamoebacastellanii. J AntimicrobChemother. 2010 Mar; 65(3), 588-91.
40. Alizadeh H, Neelam S, Cavanagh HD. Amoebicidal activities of alexidine against 3 pathogenic strains of acanthamoeba. Eye Contact Lens. 2009 Jan; 35(1):1-5.
41. Goze I, Alïm A, Dag S, Tepe B, Polat ZA. In vitro amoebicidal activity of Salvia staminea and Salvia caespitosa on Acanthamoeba castellanii and their cytotoxic potentials on corneal cells. J OculPharmacolTher. 2009 Aug; 25(4), 293-8.
42. Topalkara A, Vural A, Polat Z, Toker MI, Arici MK, Ozan F, Cetin A. In vitro amoebicidal activity of propolis on Acanthamoebacastellanii. J OculPharmacolTher. 2007 Feb; 23(1):40-55.
43. Malatyali E, Tepe B, Degerli S, Berk S, Akpulat HA. In vitro amoebicidal activity of four Peucedanum species on Acanthamoebacastellanii cysts and trophozoites. Parasitol Res. 2012 Jan; 110(1):167-74.
44. Akin Polat Z, Vural A, Tepe B, Cetin A. In vitro amoebicidal activity of four Allium species on Acanthamoebacastellanii and their cytotoxic potentials on corneal cells. Parasitol Res. 2007 Jul; 101(2), 397-402.
45. Akin Polat Z, Tepe B, Vural A. In vitro effectiveness of Thymus sipyleussubsp.Sipyleusvar.Sipyleus on Acanthamoebacastellanii and its cytotoxic potential on corneal cells. Parasitol Res. 2007 Nov; 101(6), 1551-5.

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