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The Inhibitory Effect of Poly (DMAA-co-MMA) on Bacteria, Yeast and Dermatophyte Fungi Which Cause Serious Illnesses in People

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Abstract: In this study, it was was researched the inhibitor effects of solutions including dichloromethane of poly dimethylacrylamide-co-methylmethacrylate P(DMAA-co-MMA) on microorganisms such as bacteria, yeast and dermatophyte fungi which cause serious illnesses in people. This solution, which was examined by the disc diffusion method, has antimicrobial feature upon preventing the proliferation of all bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and dermatophyte fungi except *Bacillus megaterium* and *Klebsiella pneumoniae*. In antimicrobial susceptibility data; poly P(DMAA-co-MMA) dissolved in dichloromethane has an inhibitory effect against the growth of yeast and dermatophyte fungi (on *Candida* spp. with 11.3 mm / inhibition area - 12.3 mm / inhibition area and 11.3 mm / inhibition area on *Epidermophyton* sp. - 11.3 mm / inhibition area on *Trichophyton* sp.) (P <0.001). MIC (Minimal inhibition concentration) breakpoints that strengthen the disk diffusion method are 50-100 µL (4500– 9000 µg in 10 mL) as the smallest value that inhibits the growth of bacteria, yeasts, dermatophyta. The antimicrobial compound can be of great advantage to illuminate future studies in this area. The polymer used in the study will provide a promising new addition to antimicrobial polymers that fight microorganisms that cause inflammation and fungal infections.

İnsanlarda Ciddi Hastalıklara Neden Olan Bakteri, Maya ve Dermatofit Mantarları Üzerinde Poly (DMAA-co-MMA) 'nin İnhibe Edici Etkisi

Anahtar Kelimeler
Yeni antimikrobiyal polimerik bileşik, İnhibitör etki, Poli (DMAA-co-MMA)

Öz: Bu çalışma da insanlarda ciddi hastalıklara neden olan bakteri, maya ve dermatofit mantarları gibi mikroorganizmalar üzerindeki poli dimetilakrilamid-ko-metilmetakrilat'ın poli P (DMAA-co-MMA) diklorometan içeren çözeltilerinin inhibitör etkilerini araştırıldı. Disk difüzyon yöntemi ile incelenen bu çözelti, *Bacillus megaterium* ve *Klebsiella pneumoniae* dışındaki tüm bakterilerin (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) ve dermatofit mantarlarının çoğalmasını engelleyerek antimikrobiyal özelliğe sahiptir. Antimikrobiyal duyarlılık verilerinde, diklorometan içinde çözünen poli P (DMAA-ko-MMA) maya ve dermatofit mantarlarının büyümesine karşı inhibe edici bir etkiye sahiptir (*Candida* spp. üzerinde 11.3mm / inhibisyon alanı - 12.3 mm / inhibisyon alanı) ve *Epidermophyton* sp. üzerinde 11.3 mm / inhibisyon alanı - *Trichophyton* sp. üzerinde 11.3 mm / inhibisyon alanı) (P <0.001). Disk difüzyon yöntemini güçlendiren MIC (Minimal inhibisyon konsantrasyonu) sınır değerleri bakteriler, mayalar, dermatofitlerin büyümesini engelleyen en küçük değer olarak 50-100 µL'dir (10 mL de 4500- 9000 µg). Antimikrobiyal bileşik, gelecek bu alandaki çalışmaları aydınlatmak için büyük avantaj sağlayabilir. Çalışmada kullanılan polimer, iltihaplanma ve mantar enfeksiyonlarına neden olan mikroorganizmalar ile savaşan antimikrobiyal polimerlere umut verici yeni bir katkı sağlayacaktır.

1. INTRODUCTION

In recent years, the use of polymers due to their antimicrobial properties has gained importance in scientific studies as well as in the industry [1-3]. Polymers are used efficiently and widely in antimicrobial areas due to their original properties [4]. In general, the mechanism of action of the polymers takes two forms. One of these is reactive oxygen formation on the main chain under infrared light and the other is known as the disruption of the bacterial cell membrane [5, 6]. Bacteria are developing resistance to conventional antibiotics. Therefore, it is extremely important to produce new antimicrobial materials with low bacterial resistance and high antibacterial activity. The interaction between the positive charges in the functional groups of the polymers and the negative charges of the bacterial membrane is very important [7, 8]. Cationic polymers show antimicrobial activity against bacteria and viruses because they contain many positive charges [9].

Most of the polymers having antimicrobial properties are classified as cationic quaternary polyelectrolytes and these are generally acrylate and methacrylate derivatives. Many parts of acrylate and methacrylate polymers are prepared by commercially available monomers. So as to investigate the inhibitor features of polymers, different type homo and copolymers were prepared by changing the parameters such as molecular weight, hydrophobic character and charges [10].

The activity of quaternary ammonium compounds, which are known to be biologically active, varies depending on the type of nitrogen-dependent atoms, the amount of nitrogen atoms and the counterion. The organic groups are usually aryl, alkyl and heterocyclic structures [11]. These substituents should be coherent with the hydrophobic structure of the cell wall. Therefore, it must be a hydrophobic group containing one or more long alkyl chains [12]. An increase in the alkyl chain length of a polymer has been shown to follow a rise in hydrophobic interactive relation with the lipid two layers of the cell wall, that rises the inhibitory effect of the polymer [13].

With the use of biocidal polymers, there is hope to increase the effectiveness of some existing antimicrobial compounds and also increasing their selectivity and extending the life of antibiotics [14].

Based on this statement, the development of new antimicrobials containing polymers highlights the importance of the work. Thus, we conducted the study to find out whether the polymer has an inhibitory effect on bacteria, yeast and dermatophyte fungi that cause serious diseases in humans, or whether it can be a candidate for new antimicrobial polymers. Moreover, there are no studies on the antimicrobial properties of this polymer.

2. MATERIAL AND METHODS

2.1. Screening of Antimicrobial Effects

2.1.2. Materials

Poly (dimethylacrylamide-co-methyl methacrylate); P(DMAA-co-MMA) (0.63 g) was treated in 10 mL dichloromethane (% 99.9) solvent by keeping on a rotary shaker (100 rpm) for 24 h. These materials were filtered under suitable aseptic conditions and left at 4 °C for further study. Then, 100 µL of the solutions (25 mg L^{-1}) were injected into 6 mm diameter blank antibiotic paper discs (Schleicher & Shüll No: 2668, Germany) to try the test isolates separately.

2.1.3. Microbial Strain

The bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32), yeasts (*Candida albicans* FMC 17, *Candida glabrata* ATCC 66032) and dermatophyta (*Trichophyton* sp., *Epidermophyton* sp.) were tested as species for the current study. The tested pathogens were taken by the Department of Biology, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.1.4. Sensitivity Test

The agar disc diffusion method was performed in order to detect antimicrobial sensitivity test. Mueller Hinton Agar, Yeast Malt Extract Agar and Sabouraud Dextrose Agar were prepared separately in erlen-meyer bottles under laboratory conditions and brought to 45-50 º C pouring temperature, with the culture of microorganisms to be prepared as explained, will be added at the incidence of %1 (10^6 cells mL^{-1} of bacteria, 10^4 cells mL^{-1} yeast and cells mL^{-1} dermatophyte fungi as per Mc Farland standard). 15 mL medium by shaking well is poured in to sterile petri plates and homogenously distributed. The discs (6 mm diameter) with treated 10 µL of plant solution were added to the appropriate agar media inoculated microorganism. Then, petri dishes were stored at 4 º C for 2 h. The cultivated petri dishes were incubated at 37 ± 0.1 º C at 24 h for bacterial isolates and also at 25 ± 0.1 º C at 72 h for yeasts and dermatophyte pathogens. The antibacterial, antifungal and antidermatophyte sensitivity of this polymer solution were evaluated by observing the inhibition area on the disks [15]. Micostatin and ampicillin sulbactam were used as positive control. Dichloromethane as negative control was used. The experiment was repeated twice.

2.1.5. Minimal Inhibition Concentration

MIC values of this polymer against analyzed microorganisms were revealed with a micro-well dilution method [16]. The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). The passages of microorganisms were prepared with 18-24 h broth cultures at 37±1º C for bacteria, 25 ± 0.1º C at 72 h broth cultures for yeast and

dermatophyte pathogens and they were set at a blur of 0.5 Mc Farland Standard. The polymer solution was first examined at a maximum concentration of 9000 µg and then serial 2-fold subtilizations of 6.25 to 100 µL (562.5 – 9000 µg) were performed on aseptic microtiter plates containing broth. These serial dilutions were then tested on broth cultures of bacteria, yeast, dermatophyte fungi read on an optical density meter. Microplates to be examined for growth later on were incubated for 18-24 h at 37±1ºC for bacteria and 72 h at 25±0.1ºC for yeast and dermatophyte pathogens. It was defined as the smallest value of that sample for the nominal value of the polymer used to prevent proliferation of microorganisms. This is the last tube symbolization (mg mL^{-1}) whose demectric is not microbial growth.

2.1.6. Statistical Analysis

Statistical comparisons were made between the solution and control groups (ampicillin sulbactam, micostatin) in relation to measurable preventive activity against bacteria, yeast and dermatophytes. SPSS 15 soft ware was used for statistical evaluation (SPSS Inc., Chicago IL). The values were achieved by analysis of variance (ANOVA) and the lowest significant difference (LSD) tests were specified as mean ± SE. P<0.001were evaluated for the variations between solution and control groups. P value given as foot notes below **Table 1** and **2** were considered significant effect. This study was conducted in three repetition.

3. RESULTS AND DISCUSSIONS

Polymers can be obtained from components such as fibers, films, gels, beads, nanoparticles. Polyethylene (PE), polyurethane (PU), polytetrafluoroethylene (PTFE), polyacetal (PA), polymethylmethacrylate (PMMA), polyethylenethacrylate (PET), silicone rubber (SR), polysulfone (PS), polylactic acid (PLA), polyglycolic acid (PGA) and many polymers are used in healthcare. In this study, using the polymer containing polymethylmethacrylate (PMMA) mentioned in the health field with the discovery of new antimicrobial polymers It is aimed to enable the treatment of infections caused by microorganisms [17].

Polymer solutions inhibited yeast and dermatophyte isolates and other bacteria (*E. coli*;8.6 mm / inhibition zone, *S. aureus*; 8.6 mm / inhibition zone, *P. aeruginosa*; 10.3 mm / inhibition zone) (P<0.001; d) except for *B. megaterium* and *K. penumoniae* (a: P>0.05) but compared to yeasts and dermatophytes it exhibited moderate inhibition zones (**Table 1**). Polymer solution, It showed a lower zone of inhibition on bacteria compared to ampicillin sulbactam.

The mean zone of inhibition for dermotophytes and yeasts ranged from 11.3 mm / zone of inhibition to 12.3 mm / zone of inhibition and polymer solutions showed a significant fungicidal effect notably on *C. glabrata* (11.33 mm / zone of inhibition), *Epidermophyton* sp. (11.33 mm / zone of inhibition), *Trichophyton* sp. (12.33 mm mm / zone of inhibition) control compared to that of the positive control. Mycostatin showed an effect ranging from 8.6 mm / zone of inhibition to 12.3 mm / zone of inhibition. (P<0.001; d) (**Table 1**). Negative control; dichloromethane is effective in inhibiting the growth of all microorganisms except gram negative bacteria (8.6 mm / inhibition zone). Compared to the negative control, it is varyingly effective in inhibiting the growth of all microorganisms except *B. megaterium*, *K. pneumoniae*.

Table 1. The inhibitory effects of P(DMAA-co-MMA) by the agar disc diffusion method.

Microorganisms	**P(DMAA-*co*-MMA) solutions**		
		Standart antibiotics	**DM**
E. coli	8.66±0.3[d]	12.3±0.3*	-
S. aureus	8.66±0.3[d]	10.3±0.3*	8.6±0.3
B. megaterium	-	12..3±0.3*	8.6±0.3
P. aeruginosa	10.3±0.3[d]	12.3±0.3*	-
K. pneumoniae	-	19.6±0.3*	-
C. albicans	12.3±0.3[d]	12.3±0.3**	9.6±0.3
C. glabrata	11.3±0.3[d]	8.6±±0.3**	9.6±0.3
Epidermophyton sp.	11.3±0.3[d]	8.6±0.33**	8.66±0.3
Trichophyton sp.	12.3±0.3[d]	8.6±0.33**	8.66±0.3

P(DMAA-co-MMA) solutions; P(DMAA-co-MMA) (0.63 g) was treated in 10 mL dichloromethane (% 99.9) solvent. The positive control; ampicillin sulbactam (*) and mycostatin (**) (100 µL and 20µg /disc). Dichloromethane: DM Dichloromethane as negative control was used. Inhibition zone> 15 mm (highly inhibitory effect; p<0.0001; cd), 9-14 mm (effective; P<0.001;d), (a: P>0.05): it has not inhibitory effect.

Table 2 gives the minimum inhibitory concentration of the polymer solution for complete inhibition. The initial dilution which tried to prevent the growth of the relevant microorganisms causing the disease was determined as 100 µL (9000 µg). According to this; results *C. albicans*, *Epidermophyton* sp. and the lowest inhibitor concentration on *Trcihophyton* sp. is 50 µL:4500 µg and for *C. glabrata* 100 µL; 9000 µg. On the other hand, it showed inhibitory effect with a concentration of 9000 µg effectiveness against *E. coli*, *S. aureus*, *P. aeruginosa*. The MIC values obtained for the fungi and dermatophyte species used were lower than for the bacterial species. This variability in sensitivity, We think it stems from differences in cells and cell wall structures [18]. In addition, studies on the antimicrobial effects of the polymer components (methylacrylamide and methylmethacrylate) are scarcely any.

In a study, they could not find more antimicrobial feature of poly (methacrylic acid) (PMAA) copolymers, which is one of the components of the polymer we used, and they stated that electrostatic effects owing to negatively charged surfaces only delayed bacterial binding and biofilm formation [19].

In a study done It has been reported that acrylic acid groups in copolymers have a bactericidal feature as well as its known adhesion reducing effects. It has also been stated that the high amount of acrylic acid (about 40 % by weight) in the polymer creates a hostile micro-environment for bacteria and leads to the death of microorganisms [20].

Table 2. The antimicrobial characteristic of P(DMAA-co-MMA) by the minimum inhibition concentration (MIC in 100 µL; 9000 µg).

Microorganisms	**Inhibition area (µg)**
	Poly dimethylacrylamide-comethylmethacrylate P(DMAA-co-MMA) solutions MIC values as concentration
E. coli	9000
S. aureus	9000
B. megaterium	-
P. aeruginosa	9000
K.pneumoniae	-
C. albicans	4500
C. glabrata	9000
Epidermophyton sp.	4500
Trichophyton sp.	4500

P(DMAA-co-MMA) solution that we used in our study had a bacteriostatic effect on *E. coli*, *S. aureus*, *P. aureginosa*. When the comparison is made in terms of acrylic acid groups, we think that it may have stopped the growth of bacteria instead of directly killing the bacteria due to the low amount used.

The feature that makes this study different from other studies is that; this polymer was first tested on these microorganisms and is a new drug component to be used in the pharmaceutical industry with its effectiveness on dermatophyte fungi and yeasts that cause serious diseases in humans.

In a study conducted, Amphiphilic methacrylate copolymers had a bacteriostatic effect on bactericidal on E. coli, bacteriostatic on *S. aureus* and *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp., Methacrylate copolymer had a bacteriostatic effect on *E. coli*, *S. aureus* and *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp. [21].

In one study, significant adhesion of *Proteus* sp. and *Pseudomonas* sp. was found in PMMA samples containing Gentamicin [22]. As is known, with its adhesion feature, bacteria are colonized in the environment and play an important role in pathogenesis [23]. Based on these statements and data, the PMMA substance contained in the poly-dimethylacrylamide-co-methyl methacrylate P (DMAA-co-MMA) polymer has a positive effect on the development of *P. aeruginosa*. However, according to the results of our antimicrobial susceptibility test, we can say that this polymer containing PMMA substance has an inhibitory effect on gram negative bacteria such as *P. aureginosa* by showing synergistic properties in terms of antimicrobial effect.

Polymethylmethacrylate (PMMA) has the biggest drawback promoting bacterial adhesion. In one study, *Staphylococus* strains were stated to have an adherence feature against PMMA. However, when We were performed antimicrobial susceptibility test of PMMA containing P (DMAA-co-MMA) polymer, it had a bacteriostatic effect on *S. aureus* proliferation. In this case, we can say that in terms of antimicrobial effect, the PMAA substance showed interaction with other substances and destroyed the adhesion feature of *S. aureus* [24].

The absence of any studies on dermatophytes and yeasts and the results we have obtained on dermatophytes and yeasts prove that this polymer and its ingredients can be a new antifungal polymer that can be used in the pharmaceutical field. It will shed light on the development of new polymers and in vivo applications to treat resistant microbial diseases that existing drugs can not cure.

4.CONCLUSIONS

Polymeric biomaterials are becoming increasingly important materials in different applications in the medical field due to their antimicrobial effects. Polymeric biomaterials should not cause any reaction, allergy, coagulation or inflammation when used in biomaterials in living cells and tissues [17]. In other words, the most important requirement for its use in medical applications in terms of biocompatibility is that it is compatible with the properties that determine its behavior when it comes into contact with the body [25]. The polymer can also be an alternative to usage areas in implants, biomedical devices and biological protective materials. It used in this study will provide a promising new addition to antimicrobial polymers that fight microorganisms that cause inflammation.

REFERENCES

[1] Chen H, Li M, Liu Z, Hu R, Li S, Guo Y, et al. Design of antibacterial peptide-like conjugated molecule with broad spectrum antimicrobial ability. Sci China Chem. 2018;61:113-117. Doi:10.1007/s11426-017-9034-y

[2] Peng F, Qiu L, Chai R, Meng F, Yan C, Chen Y, et al. Conjugated polymer-based nanoparticles for cancer cell-targeted and image-guided photodynamic therapy. Macromol Chem Phys. 2018;219(4):1700440. Doi:10.1002/macp.201700440

[3] Xu FJ, Neoh KG, Kang ET. Bioactive surfaces and biomaterials via atom transfer radical polymerization. Prog Polym Sci. 2009;34:719-61. Doi: 10.1016/j.progpolymsci.2009.04.005

[4] Kenawy ER, Worley SD, Broughton R. The chemistry and appli-cations of antimicrobial polymers: A state-of-the-art review. Biomacromolecules. 2007;8(5):1359-84. Doi: 10.1021/bm061150q

[5] Chemburu S, Corbitt TS, Ista L, Ji E, Lopez G, Ogawa K, et al. Light-induced biocidal action of conjugated polyelectrolytes supported on colloids. Langmuir. 2008;24(19):11053-11062. Doi: 10.1021/la8016547.

[6] Ma BC, Ghasimi S, Landfester K, Zhang KAI. Enhanced visible light promoted antibacterial efficiency of conjugated microporous polymer nanoparticles via molecular doping. J Mater Chem B. 2016; 4: 5112-5118. Doi: 10.1039/c6tb00943c.

[7] Parthasarathy A, Pappas HC, Hill EH, Huang Y, Whitten DG, Schanze KS. Conjugated polyelectrolytes with imidazolium solubilizing groups. Properties and application to photodynamic inactivation of bacteria. ACS Appl Mater Interfaces. 2015;7(51):28027-28034. Doi:10.1021/acsami.5b02771.

[8] Xu Q, He P, Wang J, Chen H, Lv F, Liu L, et al. Antimicrobial activity of a conjugated polymer with cationic backbone. Dyes Pigm. 2009;160: 519-523. Doi: 10.1016/j.dyepig.2018.08.049

[9] Murata H, Koepsel RR, Matyjaszewski K, Russell AJ. Permanent, Non-Leaching antibacterial surface2: How high density cationic surfaces kill bacterial cells. Biomaterials. 2007;28(32):4870-9. Doi: 10.1016/j.biomaterials.2007.06.012

[10] Gottenbos B, Grijpma DW, Van der Mei HC, Feijen J, Busscher HJ. Antimicrobial effects of positively charged surfaces on adhering gram-positive and gram-negative bacteria. J Antimicrob Chemother. 2001; 48:7-13. Doi: 10.1093/jac/48.1.7

[11] Li G, Shen J, Zhu Y. A Study of pyridinium-type functional polymers. III. preparation and characterization of insoluble pyridinium-type polymers. J Appl Polym Sci. 2000; 78:668-75. Doi: https://doi.org/10.1002/1097-4628(20001017)78:3<668: AID-APP230>3.0.CO;2-A

[12] Sauvet G, Dupond S, Kazmierski K, Chojnowski J. Biocidal polymers active by contact. synthesis of polysiloxanes with biocidal activity. J Appl Polym Sci. 2000; 75:1005-12. Doi: https://doi.org/10.1002/(SICI)1097-4628(20000222)75:8<1005: AID-APP5>3.0.CO;2-W

[13] Abel T, Cohen JI, Engel R, Filshtinskaya M, Melkonian A, Melkonian K. Preparation and investigation of antibacterial carbohydratebased surfaces. Carbohydr Res. 2002;337(24): 2495-9. Doi: 10.1016/s0008-6215(02)00316-6

[14] Kenawy El-R, Worley SD, Broughton R. The chemistry and applications of antimicrobial polymers: A state-of-the-art review. Biomacromolecules. 2007;8(5):1359-1384. Doi: 10.1021/bm061150q

[15] Torğut G, Pıhtılı G, Erecevit Sönmez P, Erden Y, Kırbağ S. Synthesis and antimicrobial and anticancer activities of Sodium acrylate copolymers. J Bioact Compat Poly. 2020; 35:179-188. Doi: 10.1177/0883911520913910

[16] NCCLS. Methods for dilution and antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard- fifth edition, NCCLS document M7-A5, NCCLS, Wayne, P A, USA: 2000.

[17] Tüylek Z. Smart polymers used in the field of health. J Inonu Univ Health Serv Voc Sch. 2019;7(1):81-95. Doi: 10.33715/inonusaglik.518570

[18] Ekpo MA, Etim PC. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. J Med Plant Res. 2009;3(9): 621-624.

[19] Gottenbos B, Van der mei HC, Busscher HJ, Grijpma DW, Feijen J. Initial adhesion and surface growth of *Pseudomonas aeruginosa* on negatively and positively charged poly(methacrylates). J Mater Sci Mater Med. 2000;10(12):853-5. Doi: 10.1023/a:1008989416939

[20] Gratzl G, Paulik C, Hild S, Guggenbichler JP, Lackner M. Antimicrobial activity of poly (acrylic acid) block copolymers. Mater Sci Eng C. 2014; 38:94-100. Doi: 10.1016/j.msec.2014.01.050

[21] Kamaruzzaman NF, Tan LP, Hamdan RH, Choong SS, Wong WK, Gibson AJ, et al. Antimicrobial Polymers: The potential replacement of existing antibiotics? Int J Mol Sci. 2019;20(11): 2747. Doi: 10.3390/ijms20112747.

[22] [Internet]. Anatomical structures of bacteria (Bakterilerin anatomik yapıları). [cited 2020 Febr10]. Available from: http://cdn.istanbul.edu.tr/FileHandler2.ashx?f=bakterilerin-anatomik-yapilari.pdf. 2020.

[23] Chang CC, Merritt K. Effect of *Staphylococcus epidermidis* on adherence of *Pseudomonas aeruginosa* and *Proteus mirabilis* to polymethyl methacrylate (PMMA) and gentamicin-containing PMMA. J Orthop Res. 1991;9(2):284-8. Doi: 10.1002/jor.1100090217.

[24] Veyries ML, Faurisson F, Joly-Guillou ML, Rouvei B. Control of Staphylococcal adhesion to Polymethylmethacrylate and enhancement of susceptibility to antibiotics by poloxamer. A A C. 2000;44(4):1093-1096. Doi:10.1128/aac.44.4.1093-1096.2000.

[25] Moraes Porto ICC. Polymer Biocompatibility, Polymerization. [2012 Sept 12th]. Edited by Ailton De Souza Gomes, IntechOpen. Doi: 10.5772/47786. Available from: https://www.intechopen.com/books/polymerization/polymer-biocompatibility. 2012.