Antibacterial Effect of Pre-constructed 3D Bone Scaffolds before and after Modification with Propolis

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Abstract

AIM: This study was to determine and compare the antibacterial activity of different scaffold materials before and after their modification with ethanolic extract of Egyptian propolis ethanolic extract of propolis (EEP).

SETTINGS AND DESIGN: Preparation of the dry mass of propolis, preparation of EEP, preparation of the scaffolds, and antibacterial activity testing.

MATERIALS AND METHODS: Four bacterial strains were used to determine the antibacterial activity of two different scaffold materials before and after their modification with EEP (15% and 25% by weight).

RESULTS: Tricalcium phosphate + gelatin binder modified by 25% EEP demonstrated the highest antibacterial activity against Escherichia coli. While, tricalcium phosphate + (alginate and cellulose nanowhiskers) binder modified by 25% EEP demonstrated the highest antibacterial activity Staphylococcus aureus, Streptococcus mutans, and Lactobacillus casei.

CONCLUSIONS: It can be concluded that EEP had a significant effect on the antibacterial activity of both scaffold materials; the antibacterial activity was higher against Gram-positive bacteria.

Introduction

Bone tissue engineering is a state of science and art involving bone regeneration [1]. Until the new tissue regenerates, scaffolds are considered as temporary structures that maintain the structural integrity of the tissue [2]. Today, the modern definition of a biomaterial according to the European Society for Biomaterials; material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body [3].

Ideally, a scaffold biomaterial should be; immunologically compatible, biodegradable, it should exhibit an interconnected pore structure with high porosity, and its degradation products should not cause inflammation or toxicity and must be removed from the body through metabolic pathways [1], [2].

Over the years, a lot of materials have been tested and implemented in this field [4]. Human body treats these biomaterials as foreign bodies eliciting an inflammatory and immune reactions. Bacteria will compete with cells to adhere to their surfaces, as many of them have similar mechanism of attachment as cells, except they are better adapted for survival on non-viable surfaces [4].

Incorporation of antibiotics is a common practice for preventing or treating these conditions, on the other hand, the potential risk of antibiotic resistance and the effectiveness of their long-term use are a growing concern. To meet the critical clinical need against antibacterial resistance and overcoming the long-term health implications of the current treatment strategies, there is an increased interest in the development of novel biomaterials with both intrinsic antimicrobial properties and having the potential to stimulate bone regeneration [5].

The use of natural products for curing diseases rather than depending on the conventional allopathic medicine is the current trend adopted and recommended in the field of health. There are various natural products used in the biomedical application [6].

Propolis is a natural product synthesized by honeybees, bees use it for building and preservation of their hives, killing pathogens, and preventing foreign invaders from entering the hive due to its adhesive nature [6], [7].

It is non-toxic resinous sticky substance; its chemical content depends on the geographic zone from which it comes. It has traditionally been used in curing
infections and management of numerous diseases mainly for their bacterial and viral etiologies [7].

Recently, propolis has proven its wide range of biological activities, including antibacterial, antiviral, fungicidal, anti-inflammatory, and antioxidative [8], [9]. There are distinctive types of propolis extracts; the ethanolic extract of propolis (EEP) is the most commonly used [7].

Working toward back to nature direction, this study aimed to modify scaffold materials with EEP in an attempt of developing a promising solution by constructing biomaterials combining both significant regenerative potential and enhanced antibacterial activity [5].

Accordingly, the objective of this study was to determine and compare the antibacterial activity of different scaffold materials before and after their modification with EEP.

Materials and Methods

The materials used in this study were as follows as shown in Table 1:

1. Tricalcium phosphate
2. Alginate
3. Gelatin
4. Cellulose nanowhiskers
5. Propolis

Propolis was obtained from the National Research Centre, Giza, Egypt.

6. Bacterial strains

Four bacterial strains were used in this study; Escherichia coli (MTCC443), Staphylococcus aureus (MTCC 96), Streptococcus mutans (ATCC35668), and Lactobacillus casei (ATCC 334).

7. Media used:

- LB agar used for both E. coli and S. aureus (LB agar Lennox. Batch # 135416/236)
- Brain heart infusion agar was used for S. mutans (BHI agar, LAB M. Batch # 129890/298)
- MRS agar was used for L. casei (TMMEDIAMRS Agar. Batch# M1E6ES01).

Methods

- According to the ethics guidelines, no ethical approval was needed as this article was not conducted on animals or humans
- No informed consent was needed as it was not conducted on humans.

Preparation of the dry mass of propolis

Fifty grams of propolis resin were cut into small pieces and placed in 500 ml of 70% ethanol at room temperature. The mixture of propolis resin and ethanol was then placed in an automatic shaker (W.S. Ultrasonic Mixer, Tyler, Germany). It was then placed in a rotary evaporator (EYELA Model N1001 S-W2, RIKAKIKAI Company, Tokyo) that heats and evaporates the ethanol under vacuum at 50°C until dryness.

This process will remove the ethanol and any impurities will be separated leaving a precipitated dry mass of propolis [10]. The obtained dry mass was then placed in desiccators.

Preparation of EEP for modifying the scaffold materials

The dry extracted matter was dissolved in 100 ml of 70% ethanol at room temperature and placed in an automatic shaker for 24 h. It was then filtered to obtain the EEP [10].

Preparation of the scaffolds

About 20% by weight of tricalcium phosphate particles (500 microns in size) was mixed with 80% by weight of one of two different binders; alginate and gelatin or alginate and cellulose whiskers.

- Group A: Tricalcium phosphate + (Alginate and gelatin)
- Group B: Tricalcium phosphate + (Alginate and cellulose nano whiskers)

Each group was then divided into three subgroups;

- A1: Scaffold material with no modification, A2: Scaffold material was modified by EEP (15% by weight), and A3: Scaffold material was modified by EEP (25% by weight).
- B1: Scaffold material with no modification, B2: Scaffold material was modified by EEP (15% by weight), and B3: Scaffold material was modified by EEP (25% by weight).

Antibacterial activity testing using agar disc diffusion test

The four pathogenic bacterial strains were used to determine the antibacterial activity of the six groups used in the study.

A total number of 24 (n = 24) scaffolds were cut into circular discs 1.4 cm in diameter, discs were divided into two Groups A and B (n = 12), each group was further subdivided into three subgroups (n = 4). Scaffolds were gently placed on the inoculated plates, in addition to a plate that has no disc (control plate).
Plates were then incubated at 37°C for 24 h. Zones of inhibition were determined by measuring the clear area formed around each disc the incubation period. The inhibitory zone was considered to be the shortest distance (mm) from the outer margin of the scaffold to the initial point of microbial growth [10], [11], [12]. The following test was repeated twice.

### Table 1: Materials used, batch number, brand name, and manufacturer

| Materials                  | Batch #   | Brand name      | Manufacturer         |
|----------------------------|-----------|-----------------|----------------------|
| Tricalcium phosphate       | 2018005   | Tricalcium phosphate | Nano Gate, Egypt |
| Alginate                   | 130202    | Cavex CA37       | Cavex, Holland BV    |
| Gelatin                    | Go7207173515 | Gelatin powder | PIOCHEM, Egypt |
| Cellulose nanowhiskers     | 2018004c  | Cellulose nanowhiskers | Nano Gate, Egypt |

### Statistical analysis

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. One-way ANOVA followed by Tukey post hoc tests was used to compare between more than 2 groups in non-related samples. Independent sample t-test was used to compare between two groups in non-related samples.

### Results

#### Within Group A

As shown in Tables 2-4 and Figures 1-3 E. coli, S. aureus, and L. casei; there was a statistically significant difference between the three subgroups. A statistically significant difference was found between the control A1 and each of the other two subgroups. Furthermore, a statistically significant difference was found between subgroups A2 and A3.

As shown in Table 5 and Figure 4 S. mutans; there was a statistically significant difference between the three subgroups. A statistically significant difference was found between the control A1 and each of the other two subgroups. While, between subgroups A2 and A3, there was no statistically significant difference.

#### Table 2: The mean and standard deviation (SD) values against Escherichia coli

| Variables | Escherichia coli | A | B | p-value |
|-----------|------------------|---|---|---------|
|           | Mean             | SD | Mean | SD | p-value |
| Pure material | 0.00 | 0.00 | 0.00 | 0.00 | ns      |
| With 15% propolis | 0.85 | 0.21 | 0.98 | 1.48 | 0.831* |
| With 25% propolis | 1.42 | 0.15 | 0.87 | 0.18 | <0.001* |

Significant (p<0.05), ns: Non-significant (p>0.05).

#### Between the two groups and subgroups

**E. coli** and **L. casei;** there was no statistically significant difference between subgroups A1 and B1. There was no statistically significant difference between subgroups A2 and B2. While, between subgroups A3 and B3, there was a statistically significant difference as shown in Tables 2, 4 and Figures 1 and 3.

#### Table 4: The mean and standard deviation values against Lactobacillus casei

| Variables | Lactobacillus | Gelatin | Cellulose | p-value |
|-----------|---------------|---------|-----------|---------|
|           | Mean | SD | Mean | SD | p-value |
| Pure material | 0.00 | 0.00 | 0.00 | 0.00 | 1*     |
| With 15% propolis | 1.00 | 0.14 | 1.17 | 0.44 | 0.385* |
| With 25% propolis | 1.82 | 0.12 | 2.10 | 0.14 | 0.004* |

*Significant (p<0.05), ns: Non-significant (p>0.05).

**S. aureus** and **S. mutans;** there was a statistically significant difference between the three subgroups. A statistically significant difference was found between the control B1 and each of the other two subgroups. Furthermore, between the subgroups B2 and B3, there was a statistically significant difference. As shown in Tables 3-5 and Figures 2 and 3.

#### Table 3: The mean and standard deviation values against Staphylococcus aureus

| Variables | Staphylococcus aureus | Gelatin | Cellulose | p-value |
|-----------|----------------------|---------|-----------|---------|
|           | Mean | SD | Mean | SD | p-value |
| Pure material | 0.00 | 0.00 | 0.00 | 0.00 | 1*     |
| With 15% propolis | 1.23 | 0.19 | 1.78 | 0.18 | <0.001* |
| With 25% propolis | 1.03 | 0.18 | 2.33 | 0.20 | 0.004* |

*Significant (p<0.05), ns: Non-significant (p>0.05).

**S. aureus, S. mutans;** and **L. casei;** there was a statistically significant difference between the three subgroups. A statistically significant difference was found between the control B1 and each of the other two subgroups. Furthermore, between the subgroups B2 and B3, there was a statistically significant difference. As shown in Tables 2-4 and Figures 1 and 3.

### Table 5: The mean and standard deviation values against Streptococcus mutans

| Variables | Streptococcus mutans | A | B | p-value |
|-----------|----------------------|---|---|---------|
|           | Mean | SD | Mean | SD | p-value |
| Pure material | 0.00 | 0.00 | 0.00 | 0.00 | 1*     |
| With 15% propolis | 1.82 | 0.16 | 1.07 | 0.12 | <0.001* |
| With 25% propolis | 1.92 | 0.20 | 2.00 | 0.06 | 0.362* |

*Significant (p<0.05), ns: Non-significant (p>0.05).

**S. aureus;** there was no statistically significant difference between subgroups A1 and B1. While, between subgroups A2 and B2, there was a statistically significant difference. Furthermore, between subgroups A3 and B3, there was a statistically significant difference as shown in Table 3 and Figure 2.

#### Table 6: The mean and standard deviation values of scaffolds modified with ethanolic extract of propolis against Gram-positive and Gram-negative bacteria

| Variables | Antibacterial activity of scaffolds modified with ethanolic extract of propolis | Mean | SD |
|-----------|--------------------------------------------------------------------------------|------|----|
| Gram negative | 1.029 | 0.742 |
| Gram positive | 1.681 | 0.466 |
| p-value | <0.001* |

*Significant (p<0.05), ns: Non-significant (p>0.05).
subgroups A2 and B2. Between subgroups A3 and B3, there was no statistically significant difference as shown in Table 5 and Figure 4.

Antibacterial activity of scaffolds modified with EEP against Gram-positive and Gram-negative bacteria. The antibacterial activity of the scaffolds modified with EEP against Gram positive was higher than their antibacterial activity against Gram-negative bacteria, with a statistically significant difference as shown in Table 6 and Figure 5.

Discussion

A variety of materials and manufacturing methods has been postulated to create novel alternatives to traditional bone grafts. Favorable material properties can be combined and bioactivity improved when groups of materials are used together in 3-D scaffolds [13]. For this reason in this study, composite scaffolds of tricalcium phosphate with alginate, cellulose, and gelatin binders were fabricated.

Ideally, biomaterials for bone regeneration should not only promote new tissue formation at the site of injury but also protect the wound against any related infections, which may cause prolonged inflammation and biomaterial failure [4], [14]. Thus, there is a great tendency in engineering of biomaterials to produce implants with antibacterial activity against both Gram-positive and Gram-negative bacteria [14].

Propolis is natural product; its biocompatibility has been proven combined with rare reports of allergic incidents. Its antibacterial properties can be attributed primarily to its composition as it contains different compounds including ketones, alcohols, steroids, flavonoid, phenolic acids, phenolic aldehyde, and some inorganic compounds [15].

Accordingly, a trial to go back to nature was proposed; propolis was used to modify the antibacterial activity of the two pre-3D scaffold materials.

There are different forms of propolis; ethanolic and lyophilized. The most common technique for the
production of propolis extracts is the ethanol extraction (EEP), ethanol works as a solvent resulting in obtaining low wax propolis extract, rich in biologically active components [6], [15]. The 70% ethanol was used in the present study, as it enhances the antibacterial activity by extracting most of the active constituents of propolis, moreover, 70% aqueous solution is more effective at eradication of microorganisms than absolute ethanol, because 100% ethanol denatures external membrane proteins only while water facilitates diffusion through the cell membrane [16].

In the current study, both scaffold materials were modified with EEP 15% and 25% by weight, where 25% EEP mixture is the optimum concentration as it exhibits positive significant antibacterial activity without adversely affecting the mechanical properties, in addition, increasing the EEP incorporation more than 25% weakens the scaffold and negatively affects the physical properties of the mixture; it prolongs the working time and interferes with the network formation [17].

The antibacterial activity of the tested scaffold materials was assessed by the agar diffusion test. This test allows a direct comparison of the scaffolds antibacterial effect on the microorganisms. Moreover, it is simple, rapid, reproducible and enables handling of a range of sample quantities [18].

The test was conducted against S. aureus and E. coli, the most common bacterial strains isolated from infected bone [14], [19], [20], [21]. S. mutans and L. casei, these bacterial strains were chosen due to their relevancy to surgical site infection in the oral cavity [21], [22].

The antibacterial activity of propolis should be considered on two levels; first through the direct action on the microorganism, second by stimulation of the immune system resulting in activation of natural defense of the organism. This is done through its effect on the permeability of the cellular membrane of microorganism, disruption of membrane potential, and adenosine triphosphate production as well as decreasing bacterial mobility [23].

This might explain the demonstrated antibacterial effect of EEP addition on the four types of used bacteria in both Groups A and B scaffold materials and it is supported by studies utilizing propolis proving its antibacterial activity against both Gram-positive and Gram-negative bacteria. The efficacy of propolis for inhibition of the activity of glycosyltransferase enzyme of Streptococcus circuits, S. mutans, and Streptococcus sobrinus has been confirmed in vivo and in vitro [24].

Researchers also evaluated the antibacterial activity of propolis against some anaerobic oral pathogens and confirmed that its effectiveness against Lactobacillus acidophilus, Actinomyces naeslundii, Prevotella oralis, Prevotella melaninogenica, Porphyromonas gingivalis, Fusobacterium nucleatum, and Veillonella parvula, mainly due to the presence of flavonoids and aromatic compounds such as caffeic acid in its composition [25].

It was observed in this study that the Gram-positive bacteria showed a higher mean value than that of Gram negative with a statistically significant difference. This was in accordance with Moreno et al. in 1999 [26], they concluded that propolis samples were active only against Gram-positive bacteria and some fungi. Furthermore, Sforcin et al. in 2000 [27] proved its weak activity against Gram-negative bacteria. On the other hand, Ozan et al. in 2007 [28] investigated the antibacterial effect of an experimental propolis solution, results showed a significant effect on Gram-positive strains as on Gram-negative strains. This is explained by the species-specific structure of the outer membrane of the Gram-negative bacteria and the production of hydrolytic enzymes which break down [29].

According to the results in this study, there was a significant difference between Group A and Group B regarding their antibacterial effect against the four tested bacterial species, this might be due to the high adhesion properties of the cellulose nano whiskers and the slow degradability of cellulose that makes it more difficult to attack by enzymes present in the microbial cells [30].

Conclusions

Based on the results of the following study, it can be concluded that EEP had a significant effect on the antibacterial activity of both scaffold materials; the antibacterial activity was higher against Gram-positive bacteria. In vivo studies are required to assess the immune response against tested scaffold materials.

References

1. Mohamed S, Shamaz BH. Bone tissue engineering and bony scaffolds. Int J Dent Oral Health. 2015;1(1):15-20.
2. Ercal P, Pekozer GG. A current overview of scaffold-based bone regeneration strategies with dental stem cells. In: Turksen K, editor. Cell Biology and Translational Medicine, Volume. Advances in Experimental Medicine and Biology. Vol. 1288. Cham: Springer; 2020. https://doi.org/10.1007/5584_2020_505
3. O’Brien FJ. Biomaterials and scaffolds for tissue engineering. Mater Today. 2011;14(3):88-95.
4. Griffith M, Islam MM, Edin J, Papapavlov G, Buznyk O, Patra HK. The quest for anti-inflammatory and anti-infective biomaterials in clinical translation. Front Bioeng Biotechnol. 2016;4:71. https://doi.org/10.3389/fbioe.2016.00071
PMid:27668213
5. Anastasiou AD, Nerantzaki M, Gounari E, Duggal MS, Giannoudis PV, Jha A, et al. Antibacterial properties and regenerative potential of Sr2+ and Ce3+ doped fluorapatites; a potential solution for peri-implantitis. Sci Rep. 2019;9:14469. https://doi.org/10.1038/s41598-019-50916-4
PMid:31597949

6. Khushid Z, Naseem M, Zafar MS, Najeeb S, Zohaib S. Propolis: A natural biomaterial for dental and oral healthcare. J Dent Res Dent Clin Dent Prospects. 2017;11(4):265-74.
PMid:29354255

7. Kumar LS. Propolis in dentistry and oral cancer management. N Am J Med Sci. 2014;6(8):250-9.
PMid:25006559

8. Skaba D, Morawiec T, Tanasiewicz M, Mertas A, Bobela E, Szliszka E, et al. Influence of the toothpaste with Brazilian ethanol extract propolis on the oral cavity health. Evid Based Complement Alternat Med. 2013;2013:215391. https://doi.org/10.1155/2013/215391
PMid:23861699

9. Capistrano HM, de Assis EM, Leal RM, Alvarez-Leite ME, Brener S, Bastos EM. Brazilian green propolis compared to miconazole gel in the treatment of Candida-associated denture stomatitis. Evid Based Complement Alternat Med. 2013;2013:947980. https://doi.org/10.1155/2013/947980
PMid:23737855

10. Bankova V, Popova M, Bogdanov S and Sabatini AG. Chemical composition of European propolis: Expected and unexpected results. Z Naturforsch C J Biosci. 2002;57(5-6):530-3. https://doi.org/10.1515/znc-2002-5-622
PMid:12132697

11. Aal-Saraj AB, Ariffin Z, Masudi SM. An agar diffusion study on antibacterial activity of silver-coated electrospun polycaprolactone/gelatine nanofibrous scaffolds. Sci Rep. 2020;10(1):3063. https://doi.org/10.1038/s41598-020-59391-2
PMid:32080256

12. Lim MM, Sultana N. In vitro cytotoxicity and antibacterial activity of silver-coated electrosprayed polycaprolactone/gelatine nanofibrous scaffolds. Biotechnology. 2016;6(2):211. https://doi.org/10.1007/s13205-016-0531-6
PMid:28330282

13. Turnbull G, Clarke J, Picard F, Riches P, Jia L, Han F, et al. 3D bioactive composite scaffolds for bone tissue engineering. Bioact Mater. 2017;3(3):278-314. https://doi.org/10.1016/j.bioactmat.2017.10.001
PMid:29744467

14. Przekora A. Current trends in fabrication of biomaterials for bone and cartilage regeneration: Materials modifications and biophysical stimulations. Int J Mol Sci. 2019;20(2):435. https://doi.org/10.3390/ijms20020435
PMid:30669519

15. Eskandarinia A, Kefayat A, Agheb M, Rafienia M, Baghadorani MA, Navid S, et al. A novel bilayer wound dressing composed of a dense polyurethane/propolis membrane and a biodegradable polycaprolactone/gelatin nanofibrous scaffold. Sci Rep. 2020;10(1):3063. https://doi.org/10.1038/s41598-020-59391-2
PMid:32080256

16. Margaretha I, Suniarti DF, Henda E, Mas’ud ZA. Optimization and comparative study of different extraction methods of biologically active components of Indonesian propolis Trigona spp. J Nat Prod Resour. 2012;5:233-42.

17. Beltagy TM, Abd-Elmonef ME. Antibacterial and mechanical assays of resin modified glass ionomer containing propolis extract. Egypt Dent J. 2018;64(1):33-45. https://doi.org/10.21608/edj.2018.76362

18. Wiegand C, Abel M, Ruth P, Elsner P, Hipler UC. In vitro assessment of the antimicrobial activity of wound dressings: Influence of the test method selected and impact of the pH. J Mater Sci Mater Med. 2015;26(1):5343. https://doi.org/10.1007/s10856-014-5343-9
PMid:25578697

19. Cheng T, Qu H, Zhang G, Zhang X. Osteogenic and antibacterial properties of vancomycin-laden mesoporous bioglass/PLGA composite scaffolds for bone regeneration in infected bone defects. Artif Cells Nanomed Biotechnol. 2018;46(8):1935-47. https://doi.org/10.1080/21691401.2017.1396997
PMid:29113502

20. Pant J, Sundaram J, Goudie MJ, Nguyen DJ, Handa H. Antibacterial 3D bone scaffolds for tissue engineering application. J Biomed Mater Res B Appl Biomater. 2019;107(4):1068-78. https://doi.org/10.1002/jbm.b.34199
PMid:30230685

21. Seyedmajidi S, Rajabnia R, Seyedmajidi M. Evaluation of antibacterial properties of hydroxyapatite/bioactive glass and fluorapatite/bioactive glass nanocomposite foams as a cellular scaffold of bone tissue. J Lab Physicians. 2018;10(3):265-70. https://doi.org/10.4103/jlp.jlp_167_17
PMid:30078960

22. Hameed AS, Al-Warid RJ, Obaid IA. Anti-bacterial action of multi-component bioactive glass coating for surgical suture. J Univ Babylon Pure Appl Sci. 2016;24(5):1395-400.

23. Przybyłek I, Karpinski TM. Antibacterial properties of propolis. Molecules. 2019;24(11):2047. https://doi.org/10.3390/molecules24112047
PMid:31146392

24. Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. Phytother Res. 2008;22(9):1256-63. https://doi.org/10.1002/ptr.2480
PMid:18570199

25. Abbasi AJ, Mohammad F, Bayat M, Gema SM, Ghadirian H, Seifi H, et al. Applications of propolis in dentistry: A review. Ethiop J Health Sci. 2018;28(4):505-12. https://doi.org/10.4314/ ejhs.v28i4.16
PMid:30607063

26. Moreno MI, Isla MI, Cudman NG, Vattuone MA, Sampietro AR. Screening of antibacterial activity of Amaicha Del Valle (Tucuman, Argentina) propolis. J Ethnopharmacol. 1999;68(1- 3):97-102. https://doi.org/10.1016/s0378-8741(99)00051-3
PMid:10624867

27. Siforin CM, Ferrandes A Jr., Lopes CA, Bankova V, Funari SR. Seasonal effect on Brazilian propolis antibacterial activity. J Ethnopharmacol. 2000;73(1-2):243-9. https://doi.org/10.1016/s0378-8741(99)00051-3
PMid:10624867

28. Ozan F, Sümül Z, Polat ZA, Er K, Ozan U, Deer O. Effect of mouth rinse containing propolis on oral microorganisms and human gingival fibroblast. Eur J Dent. 2007;1(4):195-200. https://doi.org/10.1055/s-0039-1698339
PMid:19212467

29. Dzierdzic A, Kubina R, Wójcicka RD, Dzik AK, Tanasiewicz M, Morawiec T. The antibacterial effect of ethanol extract of polish propolis on mutans streptococci and lactobacilli isolated from saliva. Evid Based Complement Alternat Med. 2013;2013:681891. https://doi.org/10.1155/2013/681891
PMid:23606887

30. Hickey RJ, Pelling AE. Cellulose biomaterials for tissue engineering. Front Bioeng Biotechnol. 2019;7:45.
PMid:30968018