The effect of antibacterial toothpastes on M▪c▪ococc ▪ ▪▪e ▪ 24.05.2009

Research question

What effect do antibacterial toothpastes, such as blend-a-med and Colgate, have on M▪c▪ococc ▪▪▪e ▪?

Introduction

Preliminary work revealed that both toothpastes have an antibacterial effect on M▪c▪ococc ▪▪▪e ▪. According to the preliminary study, the zone of inhibition of blend-a-med was slightly bigger than Colgate's. Therefore the antibacterial effect of blend-a-med is greater than Colgate's.

Both toothpastes contain an antimicrobial ingredient Triclosan which kills many harmful bacteria. Triclosan ultimately results in a breakdown or failure in the bacteria's cell wall. Cell wall failure affects bacteria's most vital survival processes including uptake of nutrients, inhibition of amino acid incorporation, inhibition of uracil incorporation, as well as causing membrane lysis.[1]

The antibacterial effect of toothpastes is an important factor for evaluating the benefits it has on health but it should also be considered that Triclosan and Fluoride might potentially be harmful to the user when taken in large quantities.[2]

The preliminary study also revealed that the toothpaste should be mixed with water, so that it would be well transferrable with a pipette.

(It should also be mentioned that the toothpaste which is sold under the name of "blend-a-med" in some of the European countries, is known as "Crest" in the United Kingdom.[3])

Independent variable

- The type of toothpaste used in the agar plates filled with M. ▪▪e ▪. – "Colgate Total" and "blend-a-med + eucalyptus"

Dependent variable

- Zone of inhibition on the M.▪▪e ▪ around the toothpaste in millimetres. The zone of inhibition is measured using digital callipers.

Control of variables

- The volumes of toothpaste are controlled by using a cylinder to cut out pieces of the agar

[1] http://www.ciba.com/index/ind-index/ind-per_car/ind-pc-ah/ind-pc-triclosan/ind-pc-triclosan-triclosan-101/ind-pc-triclosan-101-how-it-works.htm
[2] http://www.colgateprofessional.co.uk/products/Colgate -Total-Toothpaste/specifics
[3] http://en.wikipedia.org/wiki/Crest_(toothpaste)#cite_note -0

- The volume of agar in plates was measured and the same amount of agar was put in each plate (by a professional)
- The bacteria in each plate was distributed equally (this was controlled by a professional).
- The temperature of the plates was maintained during the investigation by keeping the plates in an incubator.

Safety issues

Although *M. luteus* is non-pathogenic and usually regarded as a contaminant, it should be considered as an emerging nosocomial pathogen in immunocompromised patients. [4] The bacteria should not be exposed to people. A lab coat and glasses should be worn to avoid the bacteria getting into the eye or on the skin. Hands should be washed after handling the agar plates. The working area should be cleaned after the experiment.

Environmental impact

The experiment does not have a significantly negative impact on the environment. . The bacteria should be sterilised to make safe after the experiment, so that it would not be in direct contact with living organisms. The agar plates and the pipettes can be recycled and reused. "Colgate" has been extensively tested and is environmentally safe[55]. The environmental impact of the toothpaste "blend-a-med" is unknown.

Hypothesis

Both products – "Colgate Total" and "blend-a-med"- note on their packages that they contain Triclosan. "Colgate Total" has 0.3% of Triclosan[6] and the exact As Triclosan is a toxic substance, both products should use about the same amount of Triclosan in the product. However neither one of the two published info about the amount of Triclosan on their web-page and the exact numbers are unknown.

How data should be recorded

Two diameters should be considered in the calculation since this gives more accurate results in calculating the difference between the two sets of data and also the variation within one set of data.

Data should be recorded in a data table, so that the exact numbers of recorded data are presented.

Standard deviation should also be calculated as this shows the variation around the mean value of data.

[4] http://en.citizendium.org/wiki/Micrococcus_luteus
[5] http://www.colgateprofessional.com/products/Colgate-Total-Advanced-Clean-Toothpaste/faqs
[6] http://lib.bioinfo.pl/pmid/journal/J%20Clin%20Dent

There will be at least 15 replicates of the experiment and the Student t-test will be used to compare the results of two sets of data and see whether there is a statistical difference between the data.

Data should also be represented on a bar chart as the results are not continuous and it would show the different results of the two toothpastes very clearly.

List of apparatus

- Mounted needle
- Marker pencil
- Ready-made 20 agar plates with M[illegible]ococc[illegible]
- Digital caliper
- Bunsen burner
- Blend-a-med + eucalypt toothpaste - 10 ml
- Colgate Total toothpaste - 10ml
- Cork borer - diameter 0,5 cm
- Two measuring cylinders - 25 ml
- 5 sterile pipettes
- An incubator
- Sterile distilled water – 20 ml

Method

1. Draw a plan to work out how many samples it is possible to fit in each plate. Taking the inhibition zones of the preliminary study into account, I decided to put four samples in each plate.
2. Label the agar plates with the symbols "B" and "C" to distinguish the types of toothpaste in each plate. There should be an equal amount of samples of both toothpastes.
3. Sterilise the working area with an antibacterial wipe and wash your hands before handling the agar plates.
4. Turn on the Bunsen burner. The flame will create updraft and it avoids bacteria falling on the plate.
5. Sterilise the cork borer in the flame.
6. Wait until the cork borer is not hot - otherwise it will melt the agar.
7. Cut uniform 'wells' in the agar plate with the cork borer.
8. If necessary, use the mounted needle to pull the cut piece out.
9. Mix both toothpastes with an equal amount of sterile distilled water in the measuring cylinder.
10. Use the pipette to transfer the two mixtures into the agar plates.

11. Change the pipette after every four plates to guarantee that the pipette is not contaminated with bacteria.
12. Repeat the procedure with each agar plate.
13. Tape all the plates up to avoid other bacteria contaminating the samples.
14. Leave the plates in an incubator at 30 degrees for 24 hours.
15. Measure the zones of inhibition so that two diameters will be recorded with the digital measurer

The effect of antibacterial toothpastes on M[illegible]cococc[illegible] [illegible]e[illegible]

Raw data

Error: +/- 0.005 mm

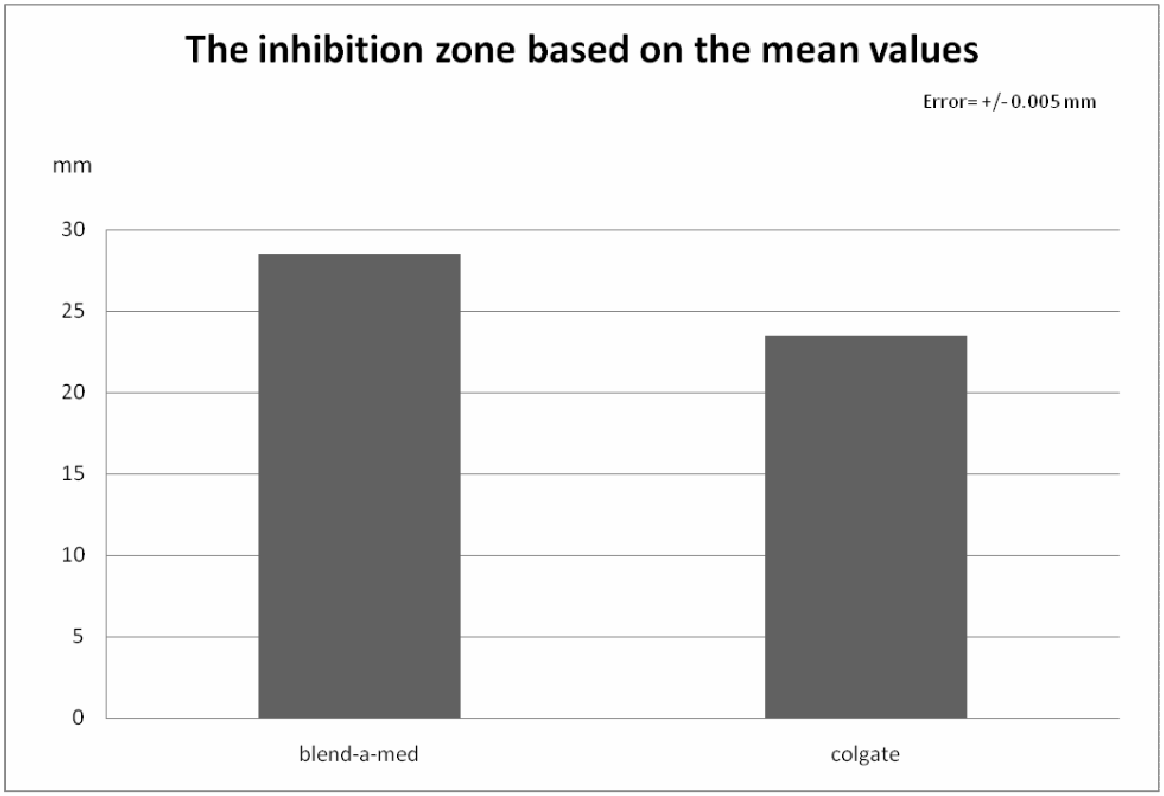
		Size of zone of inhibition in millimetres			
Number of repeat	**Diameter**	**blend-a-med**	**blend-a-med nean values of two diameters**	**Colgate**	**Colgate mean values of two diameters**
1	Diameter I	19,06	14,43	18,50	18,87
	Diameter II	19,80		19,24	
2	Diameter I	21,23	22,25	19,91	20,03
	Diameter II	23,27		20,15	
3	Diameter I	22,00	21,77	20,48	20,62
	Diameter II	21,53		20,76	
4	Diameter I	24,23	25,90	22,78	22,04
	Diameter II	27,58		21,30	
5	Diameter I	24,57	24,84	23,01	23,13
	Diameter II	25,10		23,24	
6	Diameter I	25,41	25,50	23,44	23,63
	Diameter II	25,58		23,82	
7	Diameter I	26,33	25,31	22,27	22,31
	Diameter II	24,28		22,34	
8	Diameter I	27,65	27,09	22,40	22,43
	Diameter II	28,13		22,45	
9	Diameter I	29,07	29,29	22,46	22,48
	Diameter II	29,50		22,49	
10	Diameter I	30,00	30,30	25,59	25,28
	Diameter II	30,60		24,96	
11	Diameter I	31,14	31,37	29,14	29,00
	Diameter II	31,59		28,87	
12	Diameter I	31,68	31,88	23,48	23,50

	Diameter II	32,07		23,52	
13	Diameter I	33,46	33,96	24,04	24,50
	Diameter II	34,46		24,96	
14	Diameter I	34,90	35,11	24,99	25,31
	Diameter II	35,31		25,63	
15	Diameter I	37,95	42,59	27,32	29,18
	Diameter II	47,23		31,04	

A test plate was set up which contained sterile distilled water instead of toothpaste and there was no zone of inhibition. This means that both toothpastes have an antibacterial effect and as a result the graph presents areas which are produced by the toothpaste alone.

The effect of antibacterial toothpastes on *M*[illegible]*ococc*[illegible] [illegible]*e*[illegible] with standard deviation

Graph



The mean values

The average size of the inhibition zone around "blend-a-med" was 28, 49 mm. The average inhibition zone around "Colgate" was 23, 49 mm. This means that the inhibition zones of "blend-a-med" were (28, 49 – 23, 49=) 5 mm bigger than the inhibition zones of "Colgate."

Standard deviation

The standard deviation of "blend-a-med" is 6,412845234 ≈ 6,4

The standard deviation of "Colgate" is 2,8 0016063 ≈ 2,8

The Student T-test

$$t = \frac{(28{,}49 - 23{,}49)}{\sqrt{\frac{(6{,}4)^2}{15} + \frac{(2{,}8)^2}{15}}}$$

t= 2, 772079766 ≈ **2,772**

Number of degrees of freedom

15 + 15 – 2 = 28

The probability of exceeding the critical value is **2.763**

Therefore, the two sets of data are not significantly similar.

Conclusion

"Blend-a-med" created on average 5 mm bigger inhibition zones than "Colgate"(also shown on the graph). This suggests that "blend-a-med" contains more of the antimicrobial ingredient Triclosan and the antibacterial effect of "blend-a-med" is stronger antibacterial effect on *Micrococcus lysodeikticus* than "Colgate."

Evaluation of method

The data tackle reveals that both toothpastes had larger inhibition zones in the same test plates (shown on the raw data). This is probably due to having a smaller amount of bacteria in a certain agar plate or less agar in the agar plate. The results would have been more precise if all of the test plates would contain exactly the same amount of bacteria and also the same amount of agar. However, this does not influence the difference in the effect of the toothpastes.

The amount of water which was added to the toothpaste was not measured very precisely. This might have resulted in having a smaller concentration of Triclosan in the toothpaste. According to the preliminary study and the hypothesis, both toothpastes should have had about the same sized inhibition zones. The reason why my practical did not reflect the same result might have been that "Colgate" had larger water to toothpaste ratio.

I had difficulties with accurately filling the well due to viscosity. Several times the toothpaste came out unevenly and there was not the same volume of toothpaste in each sample. I recorded the results only from the samples where there was no overspill.

I observed that each time "blend-a-med" had spilled over the edge of the 'well', the inhibition zone had changed severely while each time "Colgate" has spilled over the edge, the inhibition zone was as big as it was with samples, which had not spilled. This indicates that the concentration of Triclosan was bigger in the samples of "blend-a-med."

Evaluation of data

The standard deviation (page 7) shows that the inhibition zones of "Colgate" are more accurate than the inhibition zones of "blend-a-med." The reason for this might be that the concentration of Triclosan is larger in the samples of "blend-a-med" and the inhibition zones respond more to the toothpaste that was spilled over the specific area.

A sufficient number of repeats were made and every repeat indicated that the inhibition zone of "blend-a-med" is bigger than the inhibition zone of "Colgate." The mean values and the Student-T test also indicated that there was significant difference between the two sets of data. Therefore the conclusion is reliable.

Procedure weaknesses

The concentration of water in either toothpaste may not have been the same. Therefore the concentration of the samples was not the same. This weakness can be improved by not using water in the test or by measuring the exact volume of the toothpaste and mixing it with the same volume of water.

The application of the toothpaste can be done more carefully by squeezing the pipette from the part which is closest to the plate.

The amount of bacteria put on each plate can be measured more precisely with a pipette

The amount of agar in each plate can be measured more precisely by using a measuring cylinder.

To apply toothpaste to the plate by a pipette, it is necessary to mix toothpaste with water. The density of the toothpaste is very different from the density of water and the mixture will not be uniform. Therefore the concentration of toothpaste depends on the depth from which the sample is taken. The problem can be improved by mixing the solution continuously to get a uniform solution.

Procedure limitations

The chemical Triclosan is not present in the entire tube at the same concentration. Therefore different samples which are taken from different parts of the toothpaste tube may have a different amount of Triclosan.

References

[1] Iaconis, Michele, "How It Works", Ciba, 24.05.2010, http://www.ciba.com/index/ind-index/ind-per_car/ind-pc-ah/ind-pc-triclosan/ind-pc-triclosan-triclosan-101/ind-pc-triclosan-101-how-it-works.htm

[2] "Triclosan Poses New Danger", Nutriteam, 27.05.2010, http://www.nutriteam.com/triclo.htm

([3] http://en.wikipedia.org/wiki/Crest_(toothpaste)#cite_note -0 (further citation needed))

[4] "M▼c✓ococc ▲▼▼e ▲", Citizendium, http://en.citizendium.org/wiki/Micrococcus_luteus

[5] "Colgate Total Advanced Clean Toothpaste", Colgate, http://www.colgateprofessional.com/products/Colgate -Total-Advanced-Clean-Toothpaste/faqs

[6] "The Journal of Clinical Dentistry", BioInfoBank Library, http://lib.bioinfo.pl/pmid/journal/J%20Clin%20Dent