Non-contact electromagnetic induction heating for eradicating bacteria and yeasts on biomaterials and possible relevance to orthopaedic implant infections

IN VITRO FINDINGS

Objectives
Infection of implants is a major problem in elective and trauma surgery. Heating is an effective way to reduce the bacterial load in food preparation, and studies on hyperthermia treatment for cancer have shown that it is possible to heat metal objects with pulsed electromagnetic fields selectively (PEMF), also known as induction heating. We therefore set out to answer the following research question: is non-contact induction heating of metallic implants effective in reducing bacterial load in vitro?

Methods
Titanium alloy cylinders (Ti6Al4V) were exposed to PEMF from an induction heater with maximum 2000 watts at 27 kHz after being contaminated with five different types of microorganisms: Staphylococcus epidermidis; Staphylococcus aureus; Pseudomonas aeruginosa; spore-forming Bacillus cereus; and yeast Candida albicans. The cylinders were exposed to incremental target temperatures (35°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C) for up to 3.5 minutes.

Results
There was an average linear heating rate of 0.39°C per second up to the target temperature, and thereafter the target temperature was maintained until the end of the experiment. At 60°C and higher (duration 3.5 minutes), there was a 6-log reduction or higher for every micro-organism tested. At 60°C, we found that the shortest duration of effective induction heating was 1.5 minutes. This resulted in a 5-log reduction or higher for every micro-organism tested.

Conclusion
Non-contact induction heating of a titanium disk is effective in reducing bacterial load in vitro. These promising results can be further explored as a new treatment modality for infections of metal orthopaedic implants.

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Keywords: Infection, Periprosthetic infection, Total joint replacement, Induction heating

Article focus
- To study the feasibility and effectiveness of non-contact induction heating of a metal implant in reducing bacterial load in vitro.
- We designed an in vitro model to test the effect of heating duration and temperature of non-contact induction heating titanium cylinders inoculated with five types of micro-organisms.

Key messages
- The hypothesis is that non-contact induction heating can eradicate planktonic micro-organisms within a clinically feasible temperature range and heating duration.

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eradication of various bacterial species and yeast, including spore-forming bacteria.

- Non-contact induction heating for 1.5 minutes to 3.5 minutes at 60°C on contaminated titanium cylinders resulted in a 5-log reduction or higher of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Bacillus cereus*.

**Strengths and limitations**

- This is the first research study that determines the feasibility and effectiveness of non-contact induction heating of a metal implant in reducing bacterial load in vitro within a clinically feasible temperature range and heating duration.
- In theory, every metal implant (e.g. screw, plate, k-wire, total hip or total knee prosthesis) is eligible for induction heating, depending on anatomic relations.
- This is an in vitro study with micro-organisms in planktonic form. The influence of soft tissue, bone and biofilm have not been studied.

**Introduction**

Infection of (metal) implants is a major problem in both elective orthopaedic and acute trauma surgery, and is one of the most frequent causes of early and late failure of total knee arthroplasty.1 Although the absolute rates are low (1% to 3%),2,3 they can rise tenfold in acute trauma surgery: 20% in open tibial fractures.4 Contemporary treatment methods such as surgical debridement with pulse lavage and antibiotics may not be effective once biofilm formation has reached a certain bioburden threshold.5,6 Furthermore, the increasing resistance of bacteria to antibiotics raises concern and limits the choice of antibiotics.7 Therefore, it is paramount that alternative treatments for infections in orthopaedic implants are developed. In food preservation, heating is an effective way to reduce bacterial load.8 Furthermore, studies on hyperthermia treatment for cancer have shown that it is possible to heat metal objects within the body transcutaneously and selectively with pulsed electromagnetic fields (PEMF).9 The PEMF induces so-called ‘eddy currents’ within metallic objects10. These eddy currents are electric currents within the metallic object that oppose the change in inductance is 0 cm), there was still some distance of the coil to the titanium cylinders, which was less than 1 cm. In a clinical situation, the metal implant has a starting temperature of approximately 37°C (body temperature), so the temperature must be raised by 23° to reach 60°C. Therefore, the temperature increase of interest was 23°C.

**Patients and Methods**

Non-porous titanium cylinders (Ti6Al4V = implant alloy), of 25 mm in diameter and 13 mm in height, were exposed to a PEMF from an induction cooker (Comline 2000; Ellrona, Arnsberg, Germany) at 600 to 1000 watts (maximum power = 2000 watts) at 27 kHz after contamination with five different micro-organisms in planktonic form: *Staphylococcus epidermidis* (American Type Culture Collection (ATCC, Manassas, Virginia): 14990); *Staphylococcus aureus* (BAA:976); *Pseudomonas aeruginosa* (ATCC: 27853); spore-forming *Bacillus cereus* (ATCC: 14579); and yeast *Candida albicans* (ATCC: 10231). These micro-organisms were chosen as representatives of gram-positive bacteria, gram-negatives, spore formers and yeasts associated with infections of orthopaedic implants. The induction cooker features a pancake-type coil of 24 turns of copper litz wire with an inductance of 78 millihenries. An induction cooker was chosen because several studies have indicated that the PEMF generated by induction cookers, in the order of 20 kHz to 30 kHz, is safe for humans.13-15 For non-contact temperature measurement and temperature control, we used a calibrated infrared thermometer (IRF380-20D; Volclraft, Conrad Electronic Benelux BV, Oldenzaal, Netherlands). Figure 1 shows an arrangement of the induction system and thermal images of a heated titanium cylinder and femoral hip implant (the latter for demonstration purposes).

**Heating and distance to the induction heater.** The titanium cylinder was heated at 1000 watts (half power) at various distances from the induction heater (0 cm, 1 cm, 2 cm, 3 cm, 4 cm and 5 cm), using wooden spacers that do not interfere with the PEMF. We recorded whether the target temperature could be reached and how much time it took to reach it. When the titanium cylinder was placed on the heater without a spacer (distance is 0 cm), there was still some distance of the coil to the titanium cylinders, which was less than 1 cm. In a clinical situation, the metal implant has a starting temperature of approximately 37°C (body temperature), so the temperature must be raised by 23° to reach 60°C. Therefore, the temperature increase of interest was 23°C.

**Preparation of the cylinders.** Of each micro-organism, a 0.5 McFarlands standards (McF) suspension was made corresponding with 1.5 x 10^8 colony-forming units (CFU)
per ml. Using this solution, each cylinder was contaminated with 200 µl (0.3 x 10^8 CFU/ml). Since there are no studies available on the eradication of micro-organisms from metal implants by means of induction heating, we felt it appropriate to start with micro-organisms in planktonic form. This would allow us to investigate the possible stimulation of PEMF on micro-organisms, which may be obscured in biofilm models. It is important to note that not all micro-organisms responsible for prosthetic joint infections form biofilms. Also, induction heating may eventually be used to prevent biofilm formation (e.g. in osteosynthesis in open tibial fractures) and therefore it is paramount that we understand the effect of induction heating of metal implants infected with micro-organisms in planktonic form.

Inoculum of 0.5 Mcf, additional dilution series were made varying from 10^2 to 10^6. Samples of the dilutions were also cultured to determine the precise inoculum. From the last three dilutions, 50 µl was plated onto sheep blood agar plates (Trypticase soy agar with 5% sheep blood (bioMérieux, Marcy l’Etoile, France)). The plates were cultured for 24 and 48 hours at 37°C.

We calculated the log reduction of bacterial load using the following equation: log_{10}(A/B), where A is the number of viable micro-organisms before the experiment in CFU/ml and B is the number of viable micro-organisms after the experiment in CFU/ml.

For the calculation of the CFU, we used the results after 48 hours of incubation and the average of counted colonies from the duplicates of two experiments.

**Effect of temperature.** The cylinders were exposed different target temperatures (35°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C) for 3.5 minutes. The duration of 3.5 minutes was chosen from results in published studies with food products which revealed that 3.5 minutes caused at least a 3-log reduction of bacteria. The cylinders were placed in a Petri dish without fluid, except for control 2 (see below). Each experiment (each micro-organism and temperature) was performed in quadruplicate. After induction, the titanium cylinder was placed in a sterile container with 40 ml phosphate buffer saline (PBS). After being shaken for two minutes, the cylinder was removed. The solution was transferred to a 50 ml tube and centrifuged for 20 minutes at 8000 rpm. The supernatant was discarded and the pellet diluted in 1 ml PBS. Using this suspension, dilution series were made (10 to 10^6) in PBS. From these dilutions and the undiluted sample, 50 µl was plated onto TSS and cultured for 24 and 48 hours at 37°C.

**Effect of heating duration.** To determine the influence of heating duration, the cylinders were exposed for different time periods (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 minutes) at 60°C. This temperature was chosen because it was the lowest effective temperature for all micro-organisms. The
handling of the cylinders prior to and following induction heating was exactly the same as described above. Each experiment (each micro-organism and time) was also performed in duplicate.

**Controls.** Three conditions were used as controls. Control 1 (positive control) was a contaminated cylinder with a 200 µl micro-organism-containing suspension incubated at room temperature, without PEMf treatment and with no water surrounding the cylinder. This was the positive control.

Control 2 (PEMF control) was developed to investigate the effect of the PEMF while reducing the thermal effect: the cylinder was placed in a Petri dish with water (70 ml) to provide an environment of PEMF with water cooling. The titanium cylinders were not submerged, allowing incubation with 200 µl bacteria suspension. See Figure 2 for the temperature of control 2 during the experiment.

Control 3 (PE control) was developed to investigate the effect of material of the cylinders. These control cylinders were made of ultra-high-molecular-weight polyethylene (UHMWPE) from retrieved total knee prosthesis liners. The dimensions were the same as those from the titanium cylinders: 25 mm in diameter and 13 mm in height. The UHMWPE cylinders were also exposed to the PEMF. However, UHMWPE cannot be heated through induction heating since it does not conduct electricity.

**Statistical analysis.** Statistical analyses, when appropriate, were performed using analysis of variance (ANOVA) and regression analysis (SPSS version 23, IBM, Armonk, New York).

**Results**

**Induction heating.** Figure 2 shows the temperature profiles of each target temperature for *S. aureus*. The temperature profiles for the other bacteria were identical (data not shown). There was an average linear heating rate of 0.39°C per second (standard error 0.008 from regression analysis) up to the target temperature, and thereafter the target temperature was maintained until the end of the experiment.

**Heating and distance to the induction heater.** At 1000 watts, an increase of 23°C was reached after 23 seconds at 0 cm distance, after 37 seconds at 1 cm distance, 50 seconds at 2 cm distance, 92 seconds at 3 cm distance, 207 seconds at 4 cm distance and 592 seconds at 5 cm distance.

**Effect of temperature.** Figure 3 shows the association between temperature exposure and reduction of micro-organisms. At 60°C and higher, there was a 6-log reduction or higher for all micro-organisms. For *S. aureus*, there was a clear effect of a more than 6-log reduction at 60°C, 65°C and 70°C, although at 35°C an increase of viable bacterial counts was observed. For *P. aeruginosa*, *Candida albicans* and *S. epidermidis*, there was a clear effect of a more than 6-log reduction at 55°C, 60°C, 65°C and 70°C. For *Bacillus cereus*, a clear effect of a more than 6-log reduction was observed at 50°C, 55°C, 60°C, 65°C and 70°C.

**Effect of heating duration.** Figure 4 shows the association between duration of exposure at 60°C and reduction of micro-organisms. At 60°C for 1.5 minutes, a 5-log reduction or higher was observed for each micro-organism. For
Association between temperature exposure and reduction of micro-organisms for Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, spore-forming Bacillus cereus and yeast Candida albicans. Micro-organisms were exposed to the target temperature for 3.5 minutes. To determine the reduction, colony counts after the heating were compared with those before the heating. *Control 1: the positive control (titanium cylinder and no PEMf, see results for details) is at room temperature and plotted for each micro-organism. At 8-log reduction, the reduction of micro-organisms is total.

S. aureus, there was a clear effect of a more than 6-log reduction for 2.5, 3.0 and 3.5 minutes at 60°C. For P. aeruginosa, there was a clear effect of a more than 6-log reduction for 1.5, 2.0, 2.5, 3.0 and 3.5 minutes at 60°C. For Candida albicans, a clear effect of a more than 6-log reduction was present for 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 minutes at 60°C. For S. epidermidis, there was a clear effect of a more than 6-log reduction for 2.0, 2.5, 3.0 and 3.5 minutes at 60°C. For Bacillus cereus, the effect was steady at a 5-log reduction for 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 minutes at 60°C.

**Control conditions.** Figure 5 shows the results for the three control conditions. Except for Candida albicans, control 1 (room temperature, no PEMF) showed the
largest mean reduction of 1.1 log (95% CI -0.1 log to 2.2 log reduction) whereas control 2 (PEMf, water cooled) showed a growth of 0.6 log (95% CI 0.2 log to 1.0 log growth).

The results from control 1 were significantly different from those of control 2: difference (C1-C2) is 1.7-log reduction 95% CI 0.8 log to 2.6 log; p = 0.002. Similarly, the results of control 3 (PEMf, PE cylinder) differed significantly from those of control 2: difference (C3 to C2) is 1.2-log reduction 95% CI 0.3 log to 2.1 log; p = 0.01, except for Candida albicans. The results from control 1 did not differ from the results of control 3: difference (C1 to C3) is a 0.49-log reduction 95% CI -0.38 log to 1.36 log; p = 0.237, except for Candida albicans.

Discussion
The results of our study show that non-contact induction heating of metallic (orthopaedic) implants appears feasible and is effective in vitro to decrease the amount of living micro-organisms with log 5 or higher. Non-contact heating of the titanium cylinders was done in a predictable, controllable way, and followed a linear heating rate of 0.39°C per second. Non-contact induction heating for 3.5 minutes at 60°C, on contaminated titanium cylinders with 1.5 x 10^8 CFU/ml, resulted in a log 6 or more reduction of S. epidermidis, S. aureus, P. aeruginosa, C. albicans and B. cereus. At 60°C, we found that the shortest duration of effective induction heating was 1.5 minutes. This resulted in a 5-log reduction or higher for every micro-organism tested. For some micro-organisms such as C. albicans, induction heating at 60°C for half a minute resulted in total reduction of C. albicans CFUs.

Unexpectedly, there were more CFUs in the PEMf control (C2) than in the calibration control (C1). This effect disappeared when a PE cylinder (C3) was used, suggesting influence of material. It appears that the PEMf stimulates growth of the micro-organism on titanium when temperature conditions are favourable for bacterial growth, but too low to cause bacterial death. Del Re et al have found that exposure to PEMf at 50 Hz for 58 hours at 37°C stimulates the transposition activity and reduces cell viability of Escherichia coli. Similarly, Belyaev has shown that PEMf of 7 Hz to 11 Hz for 15 minutes acted as a non-toxic but cell growth-stimulating agent in Escherichia coli at room temperature. Although these frequencies of 7 Hz and 50 Hz are several orders of magnitude different from the 27 kHz in the present study and are not relevant for heating metal orthopaedic implants; however, they do confirm the observed stimulating effect of PEMf on the growth of micro-organisms.

The calibration control (C1) showed some reduction of CFUs without induction. We should therefore consider that titanium may demonstrate some bacteriostatic properties. This could be due to the fact that titanium forms a thin passive oxide coating of titanium(IV) oxide (TiO_2). Although passive oxide coating of TiO_2 is different from TiO_2 nanoparticles, the latter have been shown to be bactericidal with a varying degree to different types of micro-organisms.

Since contemporary treatment methods such as surgical debridement with pulse lavage and antibiotics may not be effective once biofilm formation has reached a certain bioburden threshold, induction heating may prove to be a valuable addition to these treatments.
instance, exposure to a PEMF at 72 Hz increased the effectiveness of gentamicin against the five-day biofilms of *Staphylococcus epidermidis*.20 Hajdu et al21 have shown that the antibacterial activity of antimicrobial agents is significantly enhanced by increasing the ambient temperature. Thus, the PEMF may work synergistically with thermal damage and antibiotics. These synergistic effects require further investigations, since non-contact induction heating of metal implants, when further developed, will very likely be applied in a clinical setting where antibiotics are part of the treatment strategy.

The major advantage of induction heating of metallic implants is that only the metallic implant is actively heated while induction heating has no direct heating effect on the surrounding tissue. The surrounding tissue is heated to some extent by thermal conduction from the heated metal, but if the tissue is well perfused by arterial and venous blood flow, the heat will very likely be significantly reduced as in coagulation procedures.22,23 In addition, the effect of induction heating can be focused at the implant-bone or implant-cement interface by making full use of the so-called "skin effect": with higher frequencies, the heating effect is focused at the "skin" or outer surface of the metal implant because the penetration depth of the eddy currents is only a few millimetres.10

Another advantage of induction heating is that, in theory, every metal implant, e.g. screw, plate, k-wire, total hip or total knee prosthesis, is eligible for induction heating, depending on anatomic relations. Hence, there is a potentially wide application of induction heating for treating infections.

We should consider some limitations of the current study. The results of our in vitro study apply to planktonic micro-organisms, not to micro-organisms in a biofilm. The effect of induction heating on biofilm formed on the surface of the metallic implant may be different and is subject to future studies. We found a stimulatory effect of the PEMF on bacterial growth when a titanium cylinder is placed 5 cm above the induction heater, while this took almost ten minutes with the titanium cylinder placed immediately on the induction heater. Heating metal implants deep within the human body thus requires optimisation of the induction heater and specialised designs. Nevertheless, there are already devices that can do this.9 For implants close to the skin (e.g. osteosynthesis of fractures of the ankle/foot/lower leg and hand/wrist/forearm), decrease of the PEMF with distance would be less of an issue since they are close to the coil.

It may also be challenging to heat homogeneously a metal object with an irregular shape such as the femoral stem. Homogeneous heating is important to prevent overheating of certain areas of the implant while other areas are underheated. However, this problem could be easily addressed by dividing the femoral stem in overlapping segments (of more regular shape) and heating these segments sequentially. Another advantage of using segments is that the energy required to heat a single segment to, say, 60°C is less than is required to heat the whole implant to 60°C.

In conclusion, non-contact induction heating of metallic (orthopaedic) implants appears feasible and is effective in vitro to limit survival of various bacterial species and yeast, including spore-forming bacteria. These promising results will be further explored as a new treatment modality for infections of metal orthopaedic implants.

References

1. Le DH, Goodman SB, Maloney WJ, Huddleston JI. Current modes of failure in TKA: infection, instability, and stiffness predominate. *Clin Orthop Relat Res* 2014;472:2197-2200.

2. ANJR. Australian Orthopaedic Association National Joint Replacement Registry, Annual Report, 2015. https://aoanjrr.sahmri.com/annual-reports-2015 (date last accessed 28 March 2017).

3. SHAR. Swedish Hip Arthroplasty Registry Report, 2013. http://myknee.se/pdf/SKAR2013_Eng.pdf (date last accessed 28 March 2017).
4. Prodromidis AD, Charalambous CP. The 6-hour rule for surgical debridement of open tibial fractures: a systematic review and meta-analysis of infection and nonunion rates. J Orthop Trauma 2016;30:397-402.

5. Urish KL, DeMuth PW, Craft DW, Haider H, Davis CM. Pulse lavage is inadequate at removal of biofilm from the surface of total knee arthroplasty materials. J Arthroplasty 2014;29:1128-1132.

6. Urish KL, DeMuth PW, Kwan DW, et al. Antibiotic-tolerant Staphylococcus aureus biofilm persists on arthroplasty materials. Clin Orthop Relat Res 2016;474:1649-1656.

7. Gomes DM, Ward KE, LaPlante KL. Clinical implications of vancomycin heteroresistant and intermediate susceptible Staphylococcus aureus. Pharmacotherapy 2015;35:424-432.

8. Kennedy J, Blair IS, McDowell DA, Bolton DJ. An investigation of the thermal inactivation of Staphylococcus aureus and the potential for increased thermodurability as a result of chilled storage. J Appl Microbiol 2005;99:1229-1235.

9. Huang CF, Chao HY, Chang HH, Lin XZ. A magnetic induction heating system with multi-cascaded coils and adjustable magnetic circuit for hyperthermia. Electromagn Biol Med 2016;35:59-64.

10. Cheng DK. Field and Wave Electromagnetics. Massachusetts: Addison-Wesley Publishing Company, 1991.

11. Sabale S, Jadhav V, Khot V, et al. Superparamagnetic MFe2O4 (M = Ni, Co, Zn, Mn) nanoparticles: synthesis, characterization, induction heating and cell viability studies for cancer hyperthermia applications. J Mater Sci Mater Med 2015;26:1-9.

12. Müller CW, ElKashef T, Pfeifer R, et al. Transcutaneous electromagnetic induction heating of an intramedullary nickel-titanium shape memory implant. Int Orthop 2014;38:2551-2557.

13. Koyama S, Narita E, Shinhara N, Miyakoshi J. Effect of an intermediate-frequency magnetic field of 23 kHz at 2 mT on chemotaxis and phagocytosis in neutrophil-like differentiated human HL-60 cells. Int J Environ Res Public Health 2014;11:9649-9659.

14. Miyakoshi J, Horiuchi E, Nakahara T, Sakurai T. Toxicity of TiO2 nanoparticle to denitrifying strain CFY1 and the impact on microbial community structures in activated sludge. Chemosphere 2016;144:1334-1341.

15. Li D, Li B, Wang Q, et al. Toxicity of TiO2 nanoparticle to denitrifying strain CFY1 and the impact on microbial community structures in activated sludge. Chemosphere 2016;144:1334-1341.

16. Pickering SA, Bayston R, Scammell BE. Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. J Bone Joint Surg (Br) 2003;85-B:588-593.

17. Hajdu S, Holinka J, Reichmann S, et al. Increased temperature enhances the antimicrobial effects of daptomycin, vancomycin, tigecycline, fosfomycin, and ceftamandole on staphylococcal biofilms. Antimicrob Agents Chemother 2010;54:4078-4084.

18. Hunt RW, Zavalin A, Bhatnagar A, Chinnasamy S, Das KC. Electromagnetic biostimulation of living cultures for biotechnology, biofuel and bioenergy applications. Int J Mol Sci 2009;10:4515-4558.

19. Mit D, Li B, Wang Q, et al. Toxicity of TiO2 nanoparticle to denitrifying strain CFY1 and the impact on microbial community structures in activated sludge. Chemosphere 2016;144:1334-1341.

20. Pickering SA, Bayston R, Scammell BE. Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. J Bone Joint Surg (Br) 2003;85-B:588-593.

21. Tilfotson CL, Rosenberg AE, Rosenthal DI. Controlled thermal injury of bone. Report of a percutaneous technique using radiofrequency electrode and generator. Invest Radiol 1989;24:888-892.

22. de Berg JC, Pattynama PM, Obermans WR, et al. Percutaneous computed-tomography-guided thermocoagulation for osteoid osteomas. Lancet 1996;348:350-351.

23. Matthews LS, Hirsch C. Temperatures measured in human cortical bone when drilling. J Bone Joint Surg (Am) 1972;54-A:297-308.

24. Deramond H, Wright NT, Belkoff SM. Temperature elevation caused by bone cement polymerization during vertebroplasty. Bone 1999;25:175-215.

25. Müller CW, Pfeifer R, Meier K, et al. Novel shape memory plate osteosynthesis for noninvasive modulation of fixation stiffness in a rabbit tibia osteotomy model. Biomed Res Int 2015;2015:652940.

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Author Contribution

B. G. Pijls: Research design, Acquisition of data, Analyses of data, Writing the paper. I. M. J. G. Sanders: Research design, Acquisition of data, Analyses of data, Writing the paper. E. J. Kuiper: Research design, Analyses of data, Interpretation of results, Revising the paper. R. G. H. H. Nelissen: Research design, Interpretation of results, Revising the paper

ICMJE Conflicts of Interest

None

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