A COMPARATIVE STUDY OF THE EFFECT OF HYDROGEN PEROXIDE VERSUS NORMAL SALINE ON THE STRENGTH OF THE BONE-CEMENT INTERFACE

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ABSTRACT

Hydrogen peroxide has been used for decades as an effervescent haemostatic agent in arthroplasty. Recently it has been shown to adversely affect the material properties of PMMA. We aim to assess whether any such deleterious effects are demonstrated in an experimental model which mimics the clinical use of hydrogen peroxide. Matched pairs of cancellous bone samples were treated with a swab soaked in either saline or a 3% solution of hydrogen peroxide, prior to manufacture of cement-bone constructs using Palacos or Simplex cement. Thirty pairs were then compared by subjecting them to a torsional shear force until failure and a further thirty pairs were tested to failure in tension. There was no significant difference between the mean torques to failure for the Palacos-peroxide group versus the Palacos-saline group, or the Simplex-peroxide versus the Simplex-saline group ($p=0.31$ and 0.71 respectively). Similarly there was no significant difference between the mean tension loads to failure for the Palacos-peroxide group versus the Palacos-saline group, and the Simplex-peroxide versus the Simplex-saline group ($p=0.79$ and 0.23 respectively). We conclude that the use of hydrogen peroxide as an effervescent haemostatic agent has no detrimental effect on the mechanical integrity of the bone-cement interface when compared to normal saline.

KEYWORDS: HYDROGEN PEROXIDE, BONE-CEMENT INTERFACE, ARTHROPLASTY.

INTRODUCTION

Significant research effort has been directed toward enhancing the bone-cement interface of total joint arthroplasty, in an attempt to reduce the incidence of aseptic loosening. Blood mixed in the cement reduces the hardness of the cement as well as the mechanical bond which is formed between the cancellous bone bed and the cement. In a simulated bleeding cancellous bone model, the shear strength of the cement-bone interface was reduced significantly in 50% of interfaces [1]. Hydrogen peroxide (H_20_2) has been used for decades in arthroplasty. It has three main advantageous effects: firstly, it acts as a haemostatic agent to reduce the lamination of blood products in the cement. It does this by mobilizing Ca^{2+} in platelets [2,3]. Secondly, when it comes into contact with the enzyme catalase in human erythrocytes, the following reaction occurs: $2H_20_2 \rightarrow 2H_20 + 0_2$. It is believed that the effervescence which occurs due to the production of oxygen helps to remove debris such as bone fragments, marrow or soft tissue from the cancellous bone bed [4]. Thirdly, hydrogen peroxide has a bacteriostatic action, particularly against Gram positive organisms [5].

It makes intuitive sense that a dry bone bed that is free from debris provides a superior surface at the bone-cement interface, and hydrogen peroxide helps to achieve this. However its use is not without adverse effects. A case has been reported in which cardiac arrest immediately followed its use during hip arthroplasty [6]. The most likely cause of the arrest was thought to be oxygen embolism. Several cases of non fatal embolic phenomena have also been described, particularly when hydrogen peroxide is used in enclosed spaces, such as the femoral canal. [7,8,9]. In addition, studies have also indicated that the porosity of PMMA bone cement increases by contaminating the cement with hydrogen peroxide, and that the tensile strength and yield stresses of PMMA bone cement are reduced by up to a factor of ten by contaminating samples with increasing concentrations of hydrogen peroxide [10].

This study considers the use of hydrogen peroxide as an irrigation solution in arthroplasty. One main concern is whether H_2O_2 affects the material properties of bone cement such that in the long term it contributes to aseptic loosening. This would have enormous clinical consequences, given that the reason for revision of primary total hip arthroplasty was quoted as aseptic loosening in 60.6% of cases between 1979 and 2003 in the Swedish National Hip Arthroplasty Registry (84.3% of these cases were cemented arthroplasties). Aseptic loosening was the reason for revision in 73.9% of all cases over the same period i.e. including second and subsequent revisions.

While the mechanical properties of PMMA bone cement are well documented [11-19], there is currently a paucity of published literature detailing the effect of hydrogen peroxide on the strength of the bone-cement interface. The main aim of this study is to compare the effect of introducing hydrogen peroxide with that of normal saline on the strength of a bone-cement interface by performing both tensile and torsional tests on an *in-vitro* model of a bone-cement interface using two commercially available bone cements.

MATERIALS AND METHODS

Preparation of bone-cement interface model

The materials used to prepare the bone-cement interface model were cancellous bone from a post-mortem bovine femoral greater trochanter, Palacos® bone cement with Gentamycin (Schering-Plough), Antibiotic Simplex® bone cement (Howmedica), and an irrigation solution of either normal saline or a 3% solution of hydrogen peroxide (being the concentration used clinically).

Adult proximal bovine femurs were obtained from a local abattoir (KeyPac, Co. Meath, Ireland). A portion of the greater trochanter was then sawn off with a hack-saw, and two 23mm diameter bone samples were cored out of each bovine greater trochanter using a standard coring bit. (One of each matched pair was assigned to the hydrogen peroxide group and the other to the saline group.) The cores were then attached to a diamond saw (Struers Accutom-50), from which uniform cylindrical samples of cancellous bone where obtained (see Fig. 1a). This saw cut through the bone very gently at a rate of 1mm every five seconds, under constant irrigation to minimise damage to the architecture of the cancellous bone.

The monomer and polymer for both cements were mixed together (at room temperature) using the Cemvac Ultra vacuum mixing system (vacumn of approximately -850 mbar). This apparatus was used as it has been demonstrated to result in superior mechanical properties than traditional hand mixing in an open bowl, probably as a result of reducing porosity [16]. It also results in a significantly reduced exposure for the person manufacturing the samples to MMA fumes when compared to more antiquated mixing methods [20]. Cements were mixed according to manufacturers instructions. Palacos was mixed for thirty seconds and introduced into the moulds by injection at three and a half minutes. Simplex was mixed for two minutes and applied at five minutes.

For the time interval between mixing and application**,** a swab soaked in one or other irrigation solution (either normal saline or 3% hydrogen peroxide solution) was applied to the bone surface. A 2kg weight was applied to the swabs to ensure that a good, consistent contact with the bone was maintained. The swab was then removed, and the cylindrical bone cores were placed in a custom-designed rig used to manufacture the bone-cement constructs (see Fig. 1b). The rig consists of a teflon mould and six plungers, which are removable to facilitate injection of cement onto the bone samples. Six bone cores were placed into the rig at any one time, and the mould was closed and held in a clamp. The cement was introduced in a retrograde fashion. A total weight of 3kg was then applied to the cement-bone constructs using the plunger, and the cement was allowed to cure. (This mass was chosen as it exerts a stress similar to that applied clinically, when the difference in surface area is taken into account). The constructs were removed from the mould when one hour had elapsed. Afterwards the samples diameter at the interface was reduced to 12mm using a 5mm wide lathe (see Fig. 1c). This was done to ensure that fracture occurred at the interface. At this stage samples for tensile testing were complete. For the samples to be subjected to a torsional loading the ends were finished with a milling machine to give a flat surface, suitable for the jaws of the Instron testing system – the gage section was still cylindrical (see Fig. 1d). A total of sixty proximal femurs were obtained, from which thirty bone-cement constructs were manufactured using Palacos cement, and a further thirty with Simplex.

Mechanical testing of bone-cement interface

Fifteen paired samples (hydrogen peroxide and saline solution) were tested to failure in tension and a further fifteen pairs were tested to failure in torsion for each of the bone cements. Mechanical testing was performed using an Instron FastTrack™ 8872 material testing machine that allowed both tensile and torsional loading configurations. Samples that were tested in tension had no axial pre-load applied prior to testing and were maintained in neutral rotation during testing. Testing was performed in position control and the test was run at a rate of 0.1 mm/s until failure occurred. With regard to torsional testing, again there was no pre-load applied. A twist of 0.5 degrees per second was applied to the sample in the absence of an axial force. The torque was observed to build up to a peak (which was taken as the torque to failure) before reducing gradually. Failure consistently occurred at the bone-cement interface (see Fig. 1e).

Statistical analysis

The torque and loads to failure for each sample were entered into a database for analysis (GraphPad Prism 4 statistical software package). The mean and standard deviation of each group are reported below. Paired two-tailed student's t–tests were performed to compare the means of the fifteen pairs in each experiment. The mean of the differences between the pairings is also reported, with a 95% confidence interval. Both sets of data were assessed for adequate pairing. Prism quantifies the effectiveness of pairing by calculating the Pearson correlation coefficient.

RESULTS

Palacos samples

The mean tensile load to failure for the peroxide group was 401.3 ± 126.7 N, compared with 392.7 ± 124.4 N for the saline group. The tensile load to failure for each of the fifteen paired samples is represented graphically as a scatter plot (see Fig. 2). A paired student's t-test was performed on the fifteen pairs, and this demonstrated the means not to be significantly different ($p = 0.79$). The mean difference between the pairs was 8.7 N (95% CI: -59.48 N to 76.82 N). The pairing was deemed to be significantly effective, with a correlation coefficient of 0.52 and a one-tailed p value of 0.023.

The torque to failure for the fifteen pairs is summarised graphically as a scatter plot (see Fig. 3). The mean torque to failure for the peroxide group was 2.1 ± 0.99 Nm versus 1.86 ± 0.79 Nm for the saline group. A paired student's t-test was performed of the fifteen pairs, and this demonstrated the means not to be significantly different ($p = 0.31$). The mean difference between the pairs was 0.24 Nm (95% CI: -0.2517 Nm to 0.7317 Nm). The pairing was deemed to be significantly effective, with a correlation coefficient of 0.52 and a one-tailed p value of 0.022.

Simplex samples

The mean tensile load to failure for the peroxide group was 451.3 N ± 150.2 N, compared with 492.7 N ± 116.6 N for the saline group. The tensile load to failure for each of the fifteen paired samples is represented graphically as a scatter plot (see Fig. 4). A paired student's t-test was performed of the fifteen pairs, and this demonstrated the means not to be significantly different ($p = 0.23$). The mean difference between the pairs was -41.33 N (95% CI: -111.2 N to 28.53 N). The pairing was deemed to be significantly effective, with a correlation coefficient of 0.57 and a one-tailed p value of 0.012.

The torque to failure for the fifteen pairs is summarised graphically as a scatter plot (see Fig. 5). The mean torque to failure for the peroxide group was 1.66 Nm ± 0.80 N versus 1.57 Nm

± 0.55 N for the saline group. A paired student's t-test was performed of the fifteen pairs, and this demonstrated the means not to be significantly different (p = 0.71). The mean difference between the pairs was 0.0933 Nm (95% CI: -0.4306 Nm to 0.6173 Nm). The pairing was not deemed significantly effective. An unpaired t-test on the same data reported a p value of 0.72.

DISCUSSION

Irrigating solutions, such as normal saline at room temperature, freezing normal saline, adrenaline solution, povidone iodine and hydrogen peroxide are commonly used in the preparation of bony surfaces before total joint replacement components are cemented. Their purpose is to prevent residual blood on the bone surface from laminating with the cement, as well as removing debris. Hydrogen peroxide has the added advantage of its action as a haemostatic agent. Bannister *et al.* [2] investigated the local response to freezing saline, 1:200,000 adrenaline and hydrogen peroxide: local freezing saline reduced bleeding by 24%. Saline at room temperature, adrenaline solution and hydrogen peroxide each reduced it by 14%. The effects of spinal anaesthesia (44% reduction) and of freezing saline were additive: used together they reduced bleeding by 56%. However the use of hydrogen peroxide is not without its limitations. For example, hydrogen peroxide has recently been implicated as a potential contributing factor in the development of aseptic loosening [10]. In that study, cement was contaminated during the mixing process with an unreacted hydrogen peroxide solution of either 6% or 10% concentration. The mechanical properties of the contaminated samples were then compared with uncontaminated ones, and the presence of hydrogen peroxide was found to adversely affect the fatigue life of the cement, reducing it by up to a factor of ten. The objective of this study was to determine whether the application of hydrogen peroxide to the bone surface prior to the introduction of bone cement would affect the strength of the bone-cement interface in a simplified *in vitro* model.

The experiments were designed to resemble the clinical scenario, while at the same time minimizing as much experimental variability as possible. To mimic the clinical situation, 3% hydrogen peroxide solution was applied to an actual bone-cement interface, and the hydrogen peroxide was allowed to react prior to the introduction of cement. However the current study also has also made some simplifications. Though we have tried to simulate the clinical setting, it is still an *in vitro* model based on bovine (as opposed to human) bone. An *in vivo* experiment would yield more clinically relevant results, as only then would bleeding and debris be present. Mechanically there are also differences between the *in vitro* model and reality. In the bone-cement interface model presented here, the interface has a finite geometry, unconstrained boundaries and is subject to only one mode of loading at a time, either torsion or tensile loading. In reality, the cancellous bone and cement are confined to a greater degree and are subjected to complex, three-dimenionsal loading conditions. Taking these considerations into account, the model predicts that the use of hydrogen peroxide in joint arthoplasty will not negatively affect the strength of the bone-cement interface, which might not have been expected based on previous experiments which have shown that the strength of bone cement is significantly diminished after contamination with unreacted hydrogen peroxide [10]. Had large amounts of unreacted hydrogen peroxide been present when forming the bone-cement interface, one might have expected the mechanical strength of the saline group to be greater than that of the hydrogen peroxide group due to contamination of the bone cement, however the clinical amounts used in this study do not appear to affect the strength of the interface. Using higher concentrations of hydrogen peroxide was not investigated in this study, however this is not recommended due to the increased risk of contamination.

It should be noted that the results of this study have not demonstrated a stronger mechanical bond when using hydrogen peroxide as opposed to normal saline, as has previously been reported [21]. This is possibly still the case. The reasons for this possibility include the fact that our bone samples did not actively bleed, and also there was no debris present as the bone surface had been prepared using a diamond saw. It is in the presence of these conditions where hydrogen peroxide would more likely emerge as a superior irrigation solution. In conclusion our study supports the continued use of hydrogen peroxide as an irrigation solution in arthroplasty, as it had no detrimental effect on the mechanical integrity of the bone-cement interface when compared to normal saline. Care should be taken when using it in enclosed spaces such as the femoral canal, to reduce the risk of oxygen embolism. This can be done with the use of a suction catheter in the canal while the hydrogen peroxide soaked swab is present.

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REFERENCES

[1] R.S. Majkowski, G.C. Bannister GC and A.W. Miles, The effect of bleeding on the cement-bone interface. An experimental study, *Clin Orthop* **299** (1994), 293-297.

[2] G.C. Bannister, S.K. Young, A.S. Baker, J.G. Mackinnon and P.A. Magnusson, Control of bleeding in cemented arthroplasty, *J Bone Joint Surg Br* **72** (1990), 444-446.

[3] P.C. Redondo, I. Jardin, J.M. Hernandez-Cruz, J.A. Pariente, G.M. Salido and J.A. Rosado, Effect of hydrogen peroxide on Ca2+ mobilization in human platelets, *Biochem Pharmacol* **67** (2004), 491-502.

[4] S. Mueller, H-D. Riedel and W. Stremmel, Direct Evidence for Catalase as the Predominant H_2O_2 - removing Enzyme in Human Erythrocytes, *Blood* **90** (1997), 4973-4978.

[5] P.A. Hyslop, D.B. Hinshaw, I.U. Scraufstatter, C.G. Cochrane, S. Kunz and K. Vosbeck, Hydrogen peroxide as a potent bacteriostatic antibiotic, *Free Radic Biol Med* **19** (1995), 31-37.

[6] A.J. Timperley and D.J Bracey, Cardiac arrest following the use of hydrogen peroxide during arthroplasty, *J Arthroplasty* **4** (1989), 369-70.

[7] O. Despond and P. Fiset, Oxygen venous embolism after the use of hydrogen peroxide during lumbar discectomy, *Can J Anaesth* **44** (1997), 410-3.

[8] H. Morikawa, H. Mima, H. Fujita, S. Mishima, Oxygen embolism due to hydrogen peroxide irrigation during cervical spinal surgery, *Can J Anaesth Mar* **42** (1995), 231-3.

[9] S.P. Neff, L. Zulueta and R. Miller, Hydrogen peroxide: an unusual cause of arterial and venous gas embolism, *Anaesthesia* **51** (1996), 683-4.

[10] S. Guerin, J. Harty, N. Thompson and K. Bryan, Hydrogen Peroxide as an irrigation solution in arthroplasty - a potential contributing factor in the development of aseptic loosening, *Med Hypotheses* **66** (2006), 1142-5.

[11] N.J. Dunne and J.F. Orr, Influence of mixing techniques on the physical properties of

acrylic bone cement, *Biomaterials* **22** (2001), 1819-26.

[12] P. Eyerer P and R. Jin, Influence of mixing technique on some properties of PMMA bone cement, *J Biomed Mater Res* **20** (1986), 1057-94.

[13] J. Graham, L. Pruitt, M. Ries and N. Gundiah, Fracture and fatigue properties of acrylic bone cement: the effects of mixing method, sterilization treatment, and molecular weight, *J Arthroplasty* **15** (2000), 1028-35.

[14] D. Hansen and J.S. Jensen, Mixing does not improve mechanical properties of all bone cements. Manual and centrifugation-vacuum mixing compared for 10 cement brands, *Acta Orthop Scand* **63** (1992), 13-8.

[15] E.J. Harper, W. Bonfield, Tensile characteristics of ten commercial acrylic bone cements. *J Biomed Mater Res* **53** (2000), 605-16.

[16] G. Lewis, Effect of two variables on the fatigue performance of acrylic bone cement: mixing method and viscosity, *Biomed Mater Eng* **9** (1999), 197-207.

[17] W.F. Mousa, M. Kobayashi, S. Shinzato, M. Kamimura, M. Neo, S. Yoshihara and T. Nakamura, Biological and mechanical properties of PMMA-based bioactive bone cements, *Biomaterials* **21** (2000), 2137-2146.

[18] B.P Murphy and P.J. Prendergast, The relationship between stress, porosity, and nonlinear damage accumulation in acrylic bone cement, *J Biomed Mater Res* **59** (2002), 646-54.

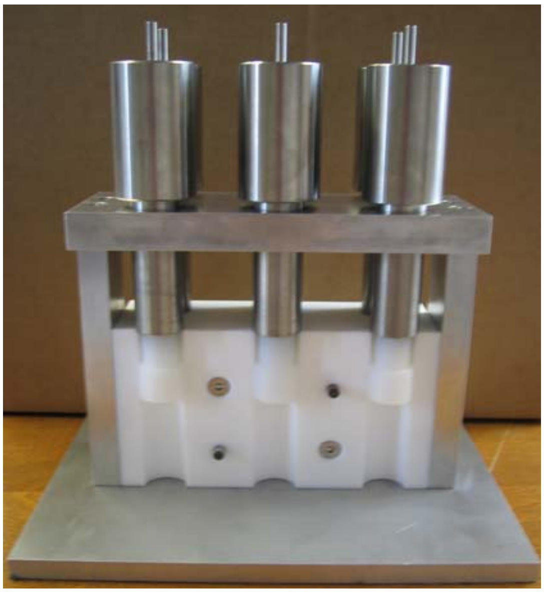
[19] B.P. Murphy and P.J. Prendergast, Multi-axial fatigue failure of orthopedic bone cement - experiments with tubular specimens, *J Mater Sci Mater Med* **14** (2003), 857-61.

[20] U.J. Schlegel, M. Sturm, V. Ewerbeck and S. Breusch, Efficacy of vacuum bone cement mixing systems in reducing methylmethacrylate fume exposure, *Acta Orthop Scand* **75** (2004), 559-566.

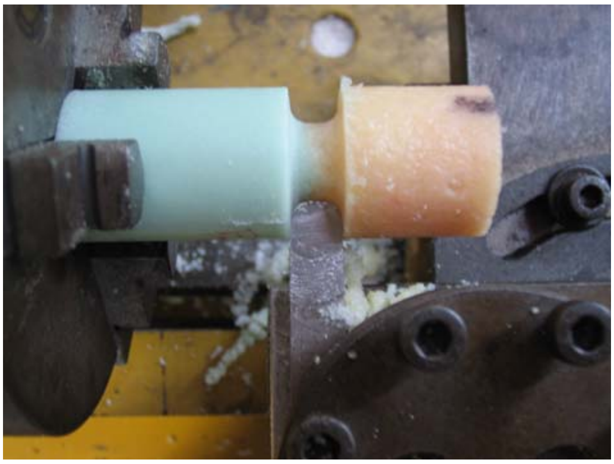
[21] R.J. Howells, J.M. Salmon, K.G. McCullough, The effect of irrigating solutions on the strength of the cement-bone interface, *Aust N Z J Surg* **62** (1990), 215-8.

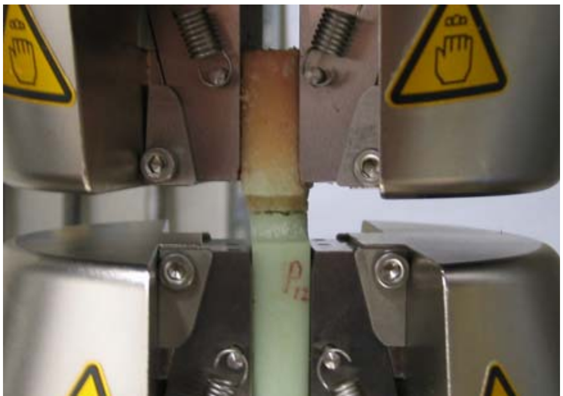
Figures

(a)



(b)





(c)

(d)

Figure 1 (a) Three pairs of finished bone samples. **(b)** Teflon mould: The mould material and plunger ends are made of Teflon. Each of the six plungers weighs 1 kg. There is a protruding rod on top to facilitate the addition of a further 2 kg. **(c)** Lathe used to create reduced diameter bone-cement interface **(d)** Sample tested to failure in torsion**:** Failure has occurred at the bone-cement interface. **(e)** Typical bone surface after tensile failure at interface.

(e)

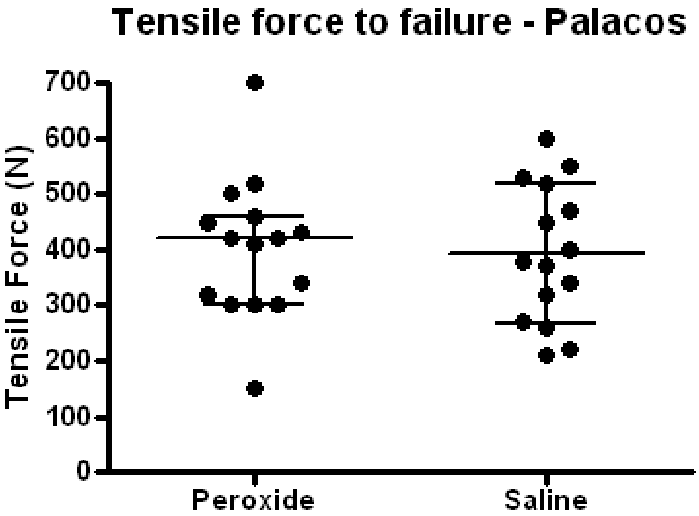


Figure 2. Tensile force (N) to failure for the fifteen pairs manufactured with Palacos-R with Gentamicin bone cement. Lines represent median and interquartile range.

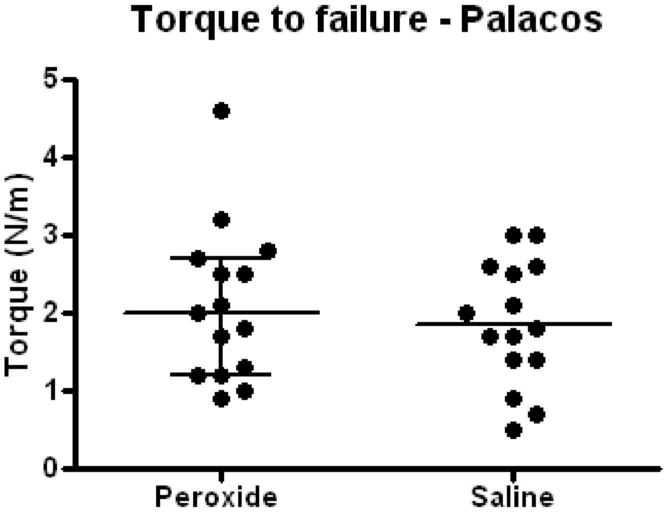


Figure 3. Torque (Nm) to failure for the fifteen pairs manufactured with Palacos-R with Gentamicin bone cement. Lines represent median and interquartile range.

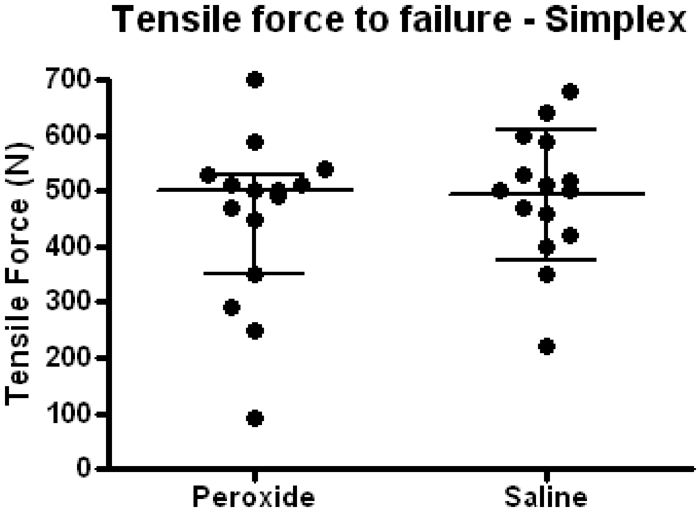


Figure 4. Tensile force (N) to failure for the fifteen pairs manufactured with Antibiotic Simplex bone cement. Lines represent median and interquartile range.

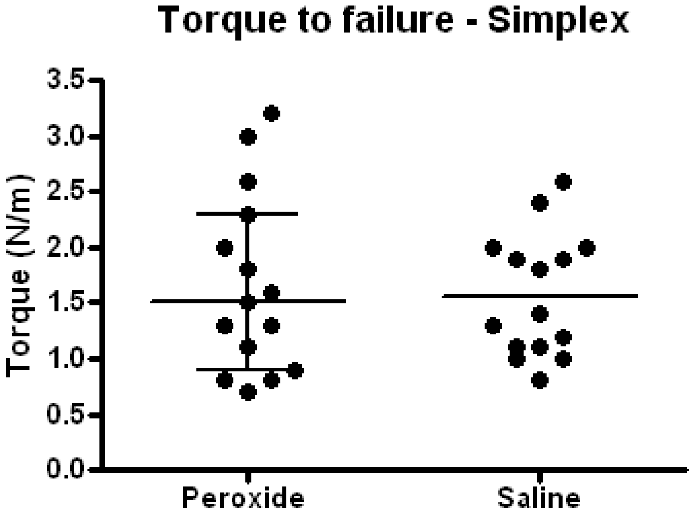


Figure 5. Torque (Nm) to failure for the fifteen pairs manufactured with Antibiotic Simplex bone cement. Lines represent median and interquartile range.