Osteoblast viability and detachment following exposure to ultrasound *in vitro*

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Ultrasound has been used in dentistry for over 40 years and has recently been proposed for cutting bone. The purpose of this study was to establish the effects of ultrasonic instruments on osteoblasts. A 25 kHz magnetostrictive ultrasound generator and a TFI-1 tip (Dentsply, UK) were used as the ultrasound generating instruments. Primary osteoblast cultures were established from the parietal bones of two-day-old Albino Wistar rats grown on tissue culture (TC) petri dishes (Corning, UK) in αMEM (Sigma, UK). Once confluent, the osteoblasts were harvested using 0.05% trypsin in 0.02% EDTA then 1.7×10^5 cells in 2.5 ml of αMEM were either re-seeded immediately onto TC dishes and allowed to adhere for 24 h or kept in suspension before application of ultrasound with different tip displacements prior to re-seeding the cells. Osteoblast viability was assessed using 0.4% Trypan Blue following the initial dose of ultrasound then periodically over a 20 h period for both adherent and suspension osteoblasts. This study demonstrated that ultrasound caused osteoblast detachment and loss of viability *in vitro*, both when adherent to a substrate or in suspension. Loss of osteoblast viability was related to the maximum displacement of the ultrasonic tip and continued throughout the 20 h period observed for osteoblasts adherent to TC dishes.

Introduction

The use of ultrasound in dentistry was originally described over 40 years ago for cavity preparation [1, 2] using an adapted industrial grinder with an abrasive slurry of aluminum oxide. The instrument efficiently removed enamel and dentine although clinicians reported impeded visibility due to the slurry. The development of the turbine high speed handpiece [3] resulted in the ultrasonic drilling instruments being phased out of use for cavity preparation and developed for other purposes. With modifications, the ultrasonic instruments were put into clinical use for the removal of calculus from the surfaces of teeth [4] and are still used extensively for this purpose.

Further adaptations to the tip used for calculus removal have led to the use of ultrasonic instruments during endodontic surgery [5, 6] for removal of root apices. No adverse effects have been reported from ultrasonic apical surgery, with good visibility maintained while using the instruments and minimal discomfort to the patient, which has led to its suggested use for cutting bone [7, 8].

Current instruments used for bone cutting include chisels, burs, lasers, pressurized water jets [9–11] and saws especially during orthopedic surgery [12]. Compared with the cut resulting from a saw, bur or chisel the precision of a cut attained using an ultrasonic drill has been suggested to be superior [7, 13]. The width of the blade of a saw is larger at the serrated edge than across the rest of the blade so the bone cut width attained from a saw will be larger than if the blade has a single chisel-shaped cutting edge and the same principle applies to the diameter of rotary burs. Ultrasonic bone cutting instruments operate by the rapid movement of a flat chisel shaped tip along one plane, it is the lack of a serrated edge and a comparatively small cutting edge that confers an increased sharpness or decreased width of the cut. A disadvantage of ultrasonic chisels is that because of the limited size of the chisel, the instruments would not be useful if large sections of bone needed to be removed. Lasers and pressurized water jets are also able to provide similar precision to the ultrasonic cutting tools [9, 10]. However, their disadvantages include thermal damage caused by lasers [9] and due to the high pressures at which the water jets operate the potential of damaging surrounding soft tissue [10] and causing surgical emphysema. A further disadvantage of the water jets is that reduced pressure of the water at a distance from the instrument limits cutting ability [11].

Earlier studies [14, 15] have suggested that healing of bone cut using ultrasound is slower in the short-term compared with bone removed using rotary burs and that the heat produced causes localized cell death although long-term histological changes were not apparent. This conflicted with later work [16, 17] that suggested no

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significant differences were apparent in the healing of bone cut with either burs or ultrasonic tools.

Materials and methods

A 25 kHz magnetostrictive ultrasound generator and a TFI-1 chisel-shaped tip (Dentsply, UK) were used as the ultrasound generating instruments for the investigation. Three different power settings, marked 1, 2 and 3 were used which corresponded with tip displacements of 9.84, 12.23 and 18.20 μm, respectively. Primary osteoblast cultures were established from stripped, minced parietal bones of two-day-old Albino Wistar rats grown on 35 mm tissue culture (TC) petri dishes (Corning, UK) in αMEM (Sigma, UK) and incubated at 37 °C in an atmosphere of 5% CO_2 and 95% humidification (Jouan IG150, France) [18].

On reaching confluence, the osteoblasts were harvested using 0.05% trypsin in 0.02% EDTA (Sigma, UK) before resuspending 1.7×10^5 cells in aliquots of 2.5 ml α-MEM and either re-seeded onto TC and allowed to reattach to the petri dishes for 24 h or kept in suspension prior to immediate exposure to ultrasound. The suspended cells were exposed to ultrasound at the three power settings in 5 ml bijouxs (Appleton Woods, UK) with five samples used for each power setting. Ensuring that the ultrasound probe did not come into contact with the sides of the bijoux it was lowered vertically 10 mm into the suspension and ultrasound applied for 30 s, the media and cells were then seeded onto 35 mm TC dishes. For the adherent osteoblast cultures, 24 h after reseeding, the αMEM was removed and replaced with fresh media to ensure that any unattached cells were removed. Once the media had been changed, the ultrasonic tip was placed vertically into the center of each of five dishes so that it was just in contact with the base of the dish, and ultrasound at three power settings, was applied for 30 s. Following exposure of the attached osteoblasts to ultrasound, all the media and detached cells were removed and re-seeded into new TC dishes. In both cases, aliquots of media and any unattached osteoblasts were removed at 0, 2, 4, 16 and 20 h to establish cell viability and the number of osteoblasts that remained unattached. Non-viable osteoblasts were demonstrated using 0.4% trypan blue and counted using a hemocytometer and readings were repeated nine times for each sample.

Statistical analysis was by ANOVA and carried out using SPSS version 10 and compared the osteoblasts that were detached from the petri dishes, with those that remain unattached with increasing power as well as the osteoblasts rendered non-viable with increasing ultrasonic power.

Results

In adherent osteoblast cultures, the number of osteoblasts that were detached from the base of the dish increased with increasing power setting and therefore tip displacement (Fig. 1). Although osteoblasts were able to reattach over 20 h for all three power settings, with increasing tip displacements the number of osteoblasts that were rendered non-viable also increased significantly $(p \leq 0.05)$ (Fig. 2), where the data compared was the

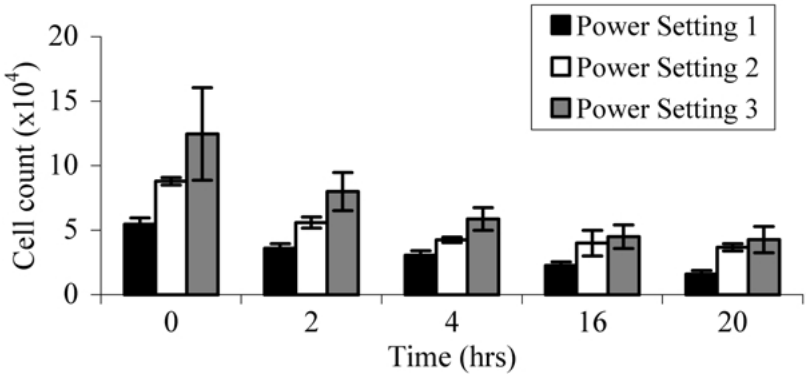

Figure 1 A graph showing the number of osteoblasts remaining unattached following exposure to ultrasound for 30 s while attached to a substrate, then reseeded into TC dishes (error bars indicate SD from mean).

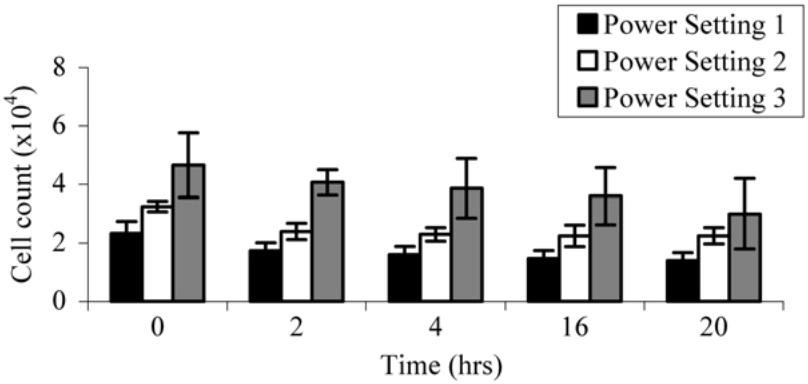

Figure 2 A graph showing the number of osteoblasts rendered non-viable following exposure to ultrasound for 30 s while attached to a substrate (error bars indicate SD from mean).

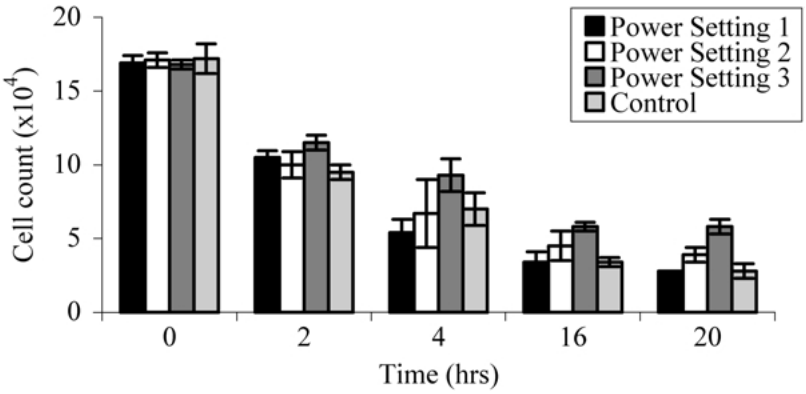

Figure 3 A graph showing the number of osteoblasts remaining unattached following exposure to ultrasound for 30 s while in suspension and reseeded into TC dishes (error bars indicate SD from mean).

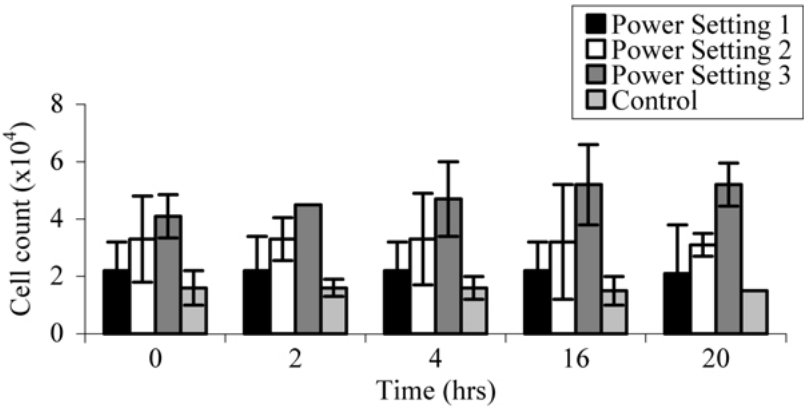

Figure 4 A graph showing the number of osteoblasts rendered non-viable following exposure to ultrasound for 30 s while in suspension (error bars indicate SD from mean).

non-viable osteoblast numbers between the three power settings. It also appeared that over the 20 h time period osteoblasts continued to be rendered non-viable (Fig. 2).

Fig. 3 shows the total number of osteoblasts that remain unattached following exposure to ultrasound while in suspension. It was noticeable that from 0 to 2 h the numbers of detached osteoblasts were comparable between all power settings, however, from 2 to 20 h there was a significant difference ($p \leq 0.05$) in the number of unattached osteoblasts between all power settings. As the power setting increased, the number of osteoblasts that remain unattached also increased significantly ($p \leq 0.05$) when comparing the osteoblast numbers between power settings and the control. When ultrasound was applied to osteoblasts in suspension, the number of cells rendered non-viable increased significantly ($p \leq 0.05$) with increasing power settings, once again the osteoblast numbers were compared between the power settings and the control. There was no noticeable change in the number of non-viable osteoblasts over the 20 h period when exposed to ultrasound in suspension.

Discussion

In order to develop an alternative bone cutting instrument it has to be shown to provide a viable alternative to the instruments in current use. It has been shown that ultrasonic cutting instruments cut slower than rotary instruments which would aid the precision of the cut [7, 13] and therefore make the cut comparable with those attained when using laser and high pressure water jets. The damage that occurs when using lasers and water jets has the potential of not remaining localized. It is possible that the lasers could cause extensive thermal damage to the underlying tissue while the bone is being cut [9]. As the original use of water jets was for cutting soft tissue [10] it has to be assumed that a considerable amount of soft tissue damage could arise if the instrument was misdirected. Although it is accepted that ultrasound will damage bone [11, 12] the extent of the damage is unclear [7, 13].

The results of this study demonstrated that ultrasound causes osteoblast detachment and loss of viability *in vitro*, both when cells were adherent to a substrate and in suspension. It is significant that the maximum displacement of the tip has an effect on the number of osteoblasts that are rendered non-viable. If the instruments are to be used for bone cutting applications osteoblasts that are affected by the ultrasound may be either damaged or non-viable. It is necessary to determine the extent of damage that is caused by the ultrasound and if the damage is temporary or permanent. The results indicated that the osteoblasts adherent to a substrate prior to application of ultrasound continue to lose viability over 20 h. The results did not show at what distance from the point of application of the ultrasound the maximum damage occurred to the osteoblasts and clearly this is important for future work to address. It is not desirable that continual damage occurs to the cells if the instruments are to be used for extensive bone cutting. It is also important to determine the zone within which maximum damage occurs. The data for viability when ultrasound

was applied in suspension suggested that the numbers of osteoblasts rendered non-viable was comparable with the number of non-viable osteoblasts when ultrasound was applied while attached to a substrate. There appeared to be no increase of non-viable osteoblasts over 20 h when ultrasound was applied in suspension which was possibly as a result of dissipation of energy within the culture medium, whilst the petri dish assisted to transmit osteoblast damaging levels of energy.

Further work is underway to assess the damage caused by the application of ultrasound directly on bone and if any changes occur in osteoblast gene expression following this treatment.

Conclusions

This study has shown that ultrasound caused damage to osteoblasts *in vitro* and that when attached to a substrate while ultrasound is applied that the cell death continues over 20 h. If an instrument is to be developed for extensive use in bone cutting applications it has to be determined to exactly what extent the ultrasound is causing damage to osteoblasts. It is necessary to determine if there is a zone of damage by the application of ultrasound directly on bone and the pattern of damage that occurs when cells are cultured on a substrate. It would also be necessary to establish the upper limit of time associated with the continual damage to osteoblasts. Further, the study demonstrated that the damage caused to osteoblasts is related to the power setting and hence the maximum tip displacement of ultrasonic instruments.

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References

1. M. CATUNA, *Ann. Dent.* **12** (1953) 256.
2. A. NIELSEN and J. RICHARDS, *J. Dent. Res.* **33** (1954) 698.
3. E. STREET, *J. Prosth. Dent.* **9** (1959) 132.
4. W. JOHNSON and J. WILSON, *J. Perio.* **28** (1957) 264.
5. M. WAPLINGTON, P. LUMLEY, A. WALMSLEY and L. BLUNT, *Endod. Dent. Traumaol.* **11** (1995) 177.
6. T. VON ARX, B. KURT, B. ILGENSTEIN and N. HARDT, *Int. Endo. J.* **31** (1998) 32.
7. I. SMALL, T OSBORN, T. FULLER, T. HUSSAIN and S. KOBERNICK, *J. Oral Surg.* **37** (1979) 159.
8. M. HONL, R. RENTZSCH, F. LAMPE, V. MULLER, O. DIERK, E. HILLE, H. LOUIS and M. MORLOCK, *Biomedizinische Technik* **45** (2000) 222.
9. J. GIRAUD, S. VILLEMIN, R. DARMANA, J. CAHUZAC, A. AUTEFAG and J. MORUCCI, *Clin. Phys. Physiol. Meas.* **12** (1991) 1.
10. E. HENRY and F. PEYTON, *J. Dental. Res.* **33** (1954) 281.
11. R. MINNS, *Clin. Materials* **10** (1992) 207.
12. B. KHAMBAY and A. WALMSLEY, *J. Dent.* **28** (2000) 31.
13. B. KHAMBAY and A. WALMSLEY, *ibid.* **28** (2000) 39.
14. T. McFALL, G. YAMANE and G. BURNETT, *J. Oral Surg.* **19** (1961) 200.
15. H. MAZOROW, *ibid.* **18** (1960) 107.
16. J. HORTON, T. TARPLEY and L. WOOD, *Oral Surg.* **39** (1975) 536.
17. J. HORTON, T. TARPLEY and L. JACOMAY, *Oral Surg.* **51** (1981) 236.
18. R. SHELTON and G. LANDINI, *Cells and Materials* **7** (1997) 147.

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