Introduction

Many new technologies with potential application to dentistry are on the horizon, including tissue engineering, gene and growth factor therapy, although there are a number of problems to overcome before these might be introduced clinically. However, the dentine-pulp complex has strong natural regenerative capacity and exploitation of these biological repair processes can allow current approaches to restorative dentistry to be optimised.

Our understanding of these biological processes has significantly advanced with appreciation that many of the cellular and molecular processes of tooth development appear to be recapitulated during dental tissue repair. Importantly, dentine seems to be far less inert than previously recognised and contains many components capable of stimulating pulpal cell responses. Growth factors, especially those of the Transforming Growth Factor-beta (TGF-β) family, are important in cellular signalling for odontoblast differentiation and stimulation of dentine matrix secretion. These growth factors are secreted by odontoblasts and deposited within the dentine matrix where they remain protected in an active form through interaction with other components of the dentine matrix. Carious demineralisation of the dental tissues can lead to their release as can application of cavity-etching agents and restorative materials like calcium hydroxide. Once released, these growth factors may play key roles in signalling many of the events of tertiary dentinogenesis, a response of pulp-dentine repair.

Consideration of how these biological processes are influenced by current restorative treatment may influence treatment outcomes. A high proportion of restored teeth show symptoms requiring endodontic treatment and 60% of all restorations are replacements for failed restorations. Thus, current restorative treatment regimens still have limitations and it is important to re-evaluate all aspects of cavity preparation and restoration to identify those factors most likely to increase the success of restorative treatment. In this review, we have investigated human pulpal responses to a range of common restorative materials and have particularly focused on factors that might influence restoration longevity. The clinical diagnosis of secondary caries is by far the most common reason for replacement of restorations, but the scientific basis for the diagnosis is meagre. However, restoration failure may arise from surgical trauma and bacterial microleakage into cavity margins. Thus, we have also examined the relationships between bacterial microleakage and restorative materials, as well as pulpal inflammation and pulp necrosis.

The importance of the remaining dentine thickness (RDT) beneath cavity preparations to pulp injury has long been recognised, although the quantitative relationships between cavity RDT, cavity dimensions, or restorative materials and pulpal injury and repair remain uncertain. Understanding and being able to predict pulpal reactions to cavity preparation and restoration events has many advantages for the practitioner, one of the most obvious being the ability to make treatment decisions which exploit the natural repair responses of the tooth. Deviations from the expected pulp responses to restorative treatment
can be used to identify potential post-operative complications more quickly and less subjectively than current indicators. This provides practitioners with the option to re-attempt restorative treatment or develop a new treatment plan before post-operative complications can progress in severity and become irreversible.

**Method**

Pulp injury and repair activities in response to individual aspects of patient factors and cavity preparation and restoration events have proved difficult to quantify, because pulp activity is the summation of all these variables. Consequently, we have examined odontoblast survival beneath the site of cavity preparation as a measure of pulp injury. This type of analysis has allowed true quantification of the degree of pulp injury, and the accuracy of this approach has made it possible to correlate pulp injury with individual cavity preparation and restorative events. We have also used area of reactionary dentine secreted as a measure of pulp repair activity. These approaches have allowed us to quantify and statistically correlate the pulpal effects of individual variables.

The analyses reported here are based on the odontometric evaluation of 410 sections of disease-free human restored teeth, from patients aged between nine and 25 years. Teeth were extracted for orthodontic or clinical reasons, and both informed patient/parental consent and ethical approval were obtained. Teeth were restored with various restorative materials, and we have focused on measuring pulp reactions, including injury, repair, bacterial microleakage and inflammation between one and 54 weeks post-operatively. Our clinical approaches have been complemented by experimental studies in cultured tooth slices to broaden the experimental conditions investigated and because of legal and ethical considerations. Pulpal injury and repair responses to cavity cutting and conditioning treatments were evaluated beneath non-exposed cavities prepared in 299 tooth slices from incisors of 28-day Wistar rats. These tooth slices were maintained in organ-culture for up to 14 days following cavity preparation treatment, prior to histological assessment of odontoblast survival and reactionary dentine area. In quantifying these pulpal reactions, we have performed analysis of variance (ANOVA) statistical correlations with cavity preparation, restoration events and patient variables to provide guidelines on pulp activity following treatment.

**Results and Discussion**

**Pulp injury**

Odontoblast survival beneath cavity preparations was used to quantify pulp injury. Our attention was focused on odontoblast survival, because these cells regulate dentine synthesis, secretion and mineralisation throughout life. While the two other major pulp cell populations, the cells of the subodontoblast layer and the fibroblasts of the pulp core, are important in supporting odontoblast activity, they do not appear to play a direct role in the secretion of dentine matrix.

Odontoblast survival appeared most sensitive to cavity RDT, cavity preparation in the absence of coolant, and restoration materials. Odontoblast survival was least influenced by other cavity preparation and restoration variables (Table 1). The sequence of correlation between treatment variables and reductions in odontoblast survival suggests that pulp vitality is most likely to be maintained by concentrating attention on maximising RDT and using adequate coolant during cavity preparation in addition to selecting the most appropriate material for cavity restoration. For cavity etching, optimal dentine condition is achieved with 60s EDTA treatment to maximise odontoblast survival and reactionary dentine (Figure 1). Either avoidance or over-use of etching, seems to result in suboptimal odontoblast survival and reactionary dentine repair activity (Figure 1). The ability of cavity etchants to dissolve growth factors from the dentine matrix may reflect the influence of these agents in stimulating reactionary dentineogenesis.

**Cavity preparation treatments**

Cavity RDT provides pulp protection from the injurious effects of trauma produced during cavity cutting. Heat has been suggested to be the most
injurious effect of cavity cutting.\textsuperscript{20} Intrapulpal heat generated during cavity preparation is determined by the drill rotation speed,\textsuperscript{21} size, type and shape of the cutting instrument,\textsuperscript{22} length of the time the instrument is in contact with dentine,\textsuperscript{23} the amount of pressure exerted on the handpiece,\textsuperscript{24} cutting technique and the use of coolants.\textsuperscript{25} Our findings show that the use of adequate coolant, directed at the point of cutting, is a critical factor in preventing avoidable pulp injury. Beneath cavities cut in the absence of coolant at 8000 rpm, the survival of odontoblasts was reduced by 48\%, in comparison with similar cavities cut under coolant (Figure 1). Cavity cutting with coolant at different bur speeds reduced odontoblast survival by 36\% (500 rpm), 28\% (4000 rpm), and 34\% (20,000 rpm) in comparison with a 8000 rpm bur speed (Figure 1). The airotor handpiece generates an 8000 rpm bur speed and other bur speeds can be selected using electric handpieces. Information on the effects of higher bur speeds is lacking but might be expected to cause similar levels of odontoblast injury. However, the significance of bur speeds on pulp injury was not as great as the requirement to use coolant, or minimise iatrogenic removal of dentine to maximise RDT (Table 1).

**Reactionary dentine**

Dentine matrix secretion can be classified as either primary, secondary or tertiary in origin according to the chronology and circumstances of its secretion by odontoblasts. If pre-existing odontoblasts survive the trauma of primary and secondary dentine injury, these cells are stimulated to secrete new tertiary dentine matrix, termed reactionary dentine. Measurement of reactionary dentine area provides a measure of pulp repair activity.\textsuperscript{13} Reactionary dentine secretion was influenced by the absence of coolant, bur speed during cutting, cavity etching, cavity RDT, cavity wall depth, cavity volume and restoration material, but the cavity surface area appeared to have little effect (Table 1). This evidence suggests that almost all the cavity preparation and restoration variables can influence pulp repair activity. A number of these variables will influence the release of bio-active molecules from the dentine matrix and their capacity to stimulate repair processes.

**Cavity remaining dentine thickness**

Dentine protection of the pulp tissue can create large differences in pulp responses to the cutting of shallow or deep cavity preparations. Deep cavities with small RDTs leave the pulp tissue less protected from preparation trauma, and also from the chemical activity of dental materials, while the pulp beneath shallow cavities is better protected against injury. Over the years, estimates of the minimal cavity RDT which does not cause unequivocal pulp injury have been decreasing. In 1984, Stanley\textsuperscript{26} suggested that an RDT of 2 mm would protect the pulp from most restorative procedures. Subsequently in 1991, Pameijer et al\textsuperscript{27} reported that an RDT of 1 mm or more would protect the pulp tissue from the cytotoxic effects of zinc phosphate and resin-modified glass ionomer materials. Recently, it has been suggested that these were cautious estimates, and deeper preparations carefully cut down to 0.5 mm appeared to have only limited effect on underlying odontoblast survival.\textsuperscript{13} To aid practitioner appreciation of pulp dentine responses to the RDT of preparation forms, we have shown these schematically in Figure 2.

Cavity RDT was an important mediator of pulp responses to pulp injury and pulp repair (Table 1) as well as pulp inflammation (Figure 3).
The severity of pulp inflammation was found to be greater in deeper cavity preparations, particularly in the presence of bacterial microleakage (Figure 3). Most severe forms of inflammation and necrosis were observed in cavities containing bacteria with an RDT between 0.25-0.004 mm (Figure 3). Odontoblast survival was maximal in shallow cavity preparations with an RDT between 3.0-1.01 mm, in the absence of bacteria (Figure 3), while approximately 48% of odontoblasts were found to be injured following the preparation of deep cavities with small (0.25-0.004 mm) RDTs (Figure 3). This would suggest that the preparation of deep cavities (with an RDT between 0.25-0.004 mm) injures underlying odontoblasts, preventing the secretion of reactionary dentine beneath these very deep cavity preparations. Shallower cavity preparation may cause only limited cellular damage and yet still allow diffusion down the dentinal tubules of growth factors and other bio-active molecules released from the dentine matrix. Interaction of these molecules with the odontoblasts could then lead to signalling of reactionary dentino genesis. However, in very shallow cavities, the extent of reactionary dentino genesis seems to decrease. This may well be related to the limited release of growth factors and other bio-active molecules during cavity preparation and their ability to diffuse only finite distances down the dentinal tubules to interact with the odontoblasts. These observations provide a guide to assessing pulp activity following restorative treatment and deviation from these responses may aid the identification of potential complications.

Restorative materials
Selecting between different types of restorative materials to optimise treatment outcomes on the basis of limited scientific data often proves problematical for practitioners. Information on the success of materials is normally based on longevity surveys. Often the reason for replacement of restorations is poorly characterised, and it is not possible to understand how cavity preparation and restoration events, in addition to pulp reactions, contributed to the development of complications and eventual restoration failure. The methods presented here show how individual cavity preparation and restoration events and patient factors can be correlated to odontoblast numbers and pulp injury, reactionary dentine repair activity and bacterial microleakage (Table 1).

Following cavity restoration, the area of reactionary dentine appeared to be correlated with different restorative materials and cavity RDT (Figure 4). Calcium hydroxide was associated with the greatest area of reactionary dentine, while no reactionary dentine deposition was observed following cavity restoration with zinc polycarboxylate (Figure 4). Much greater quantities of reactionary dentine were secreted with RDTs below 0.5 mm, except with zinc oxide eugenol (Figure 4). Composite resin, enamel-bonded composite resin, and resin-modified glass ionomer were associated with intermediate levels of reactionary dentine secretion (Figure 4). These observations may be explained by the possible cytotoxic effects of components leached from these materials and their involvement in releasing bio-active signalling molecules from the dentine matrix.8

Cavity RDT exacerbated the effects of materials which were injurious to underlying odontoblast numbers, such as enamel-bonded
Composite resin and resin-modified glass ionomers, where reductions were approximately 60% with an RDT between 0.5 and 0.004 mm in comparison with an RDT between 3.0 and 0.5 mm (Figure 4). In contrast, odontoblast numbers were largely maintained beneath restorations restored with calcium hydroxide (Figure 4). Intermediate degrees of odontoblast survival were observed for polycarboxylate, zinc oxide eugenol, and composite resin materials (Figure 4).

It is clear that pulp responses are the summation of multifactorial events, however the effect of individual variables are not uniform and this explains why we have presented each of the tables as a sequence (Table 1). We postulate that the concentration of practitioner attention on the more important restoration variables is likely to lead to the most productive improvements in treatment outcomes.

Bacterial microleakage

Microleakage complications include post-operative sensitivity, marginal discolouration, periodontal disease, recurrent caries, inflammation, necrosis, and the eventual need for endodontic therapy. Different restorative materials were observed to have an important influence on bacterial microleakage. Zinc oxide eugenol and resin-modified glass ionomer were found to prevent bacterial microleakage into 100% of cavity restorations for up to one year following surgery (Figure 4).

These observations can be attributed to antibacterial activity and direct sealing with cavity walls. However, the placement of enamel-bonded composite resin and adhesive-bonded composite resin did not appear to result in a perfect seal with cavity walls, with bacteria being detected in 22% and 10% respectively of these restorations (Figure 4). These observations confirm earlier reports that zinc oxide eugenol restorations have significantly lower bacterial counts than other comparable dental materials. The favourable sealing characteristics of resin-modified glass ionomer explain why it is particularly recommended for Class V cavities in caries-prone patients. The detection of bacteria in composite resin restorations restored with calcium hydroxide (Figure 4) demonstrates the continuing need to make improvements to the adherence and marginal sealing of these materials, by using sand-blasting placement techniques to reduce shrinkage during polymerisation. The greater prevalence of bacteria in restorations following use of enamel-bonded composite resin suggests that inadequate bonding to dentine may allow increased microleakage of bacteria through cavity margins. We lack data on the bacterial microleakage performance of calcium hydroxide, but it has been reported in non-human primates that this is approximately 9.4% due to an inability to completely seal with cavity margins and a lack of sustained antibacterial activity. These observations demonstrate the importance of restorative material selection on pulp injury and repair activity, and how these might influence restoration complications and longevity.

Pulp inflammation

Inflammatory reactions can injure the pulpal cell population and lead to pulp complications in response to cavity restorations, which may initially appear to be successful. Severe forms of inflammatory activity can develop into total pulpal necrosis, and periapical lesion development with local bone destruction. In less severe cases, the inflamed pulp is associated with hypersensitivity, so that thermal, mechanical or osmotic stimuli encountered in normal function can cause intense pain. These observations explain why inflammatory activity is associated with the high rates of primarily vital teeth exhibiting endodontic complications following cavity restoration. Pulp inflammation was the most highly correlated variable with bacterial microleakage, followed by the selection of restorative material, and the time elapsed since surgery (Table 1). Other variables, such as reactionary dentine secretion, odontoblast survival, cavity RDT and acid-etching, were less important (Table 1). Consequently, cavities restored with materials permitting higher rates of bacterial microleakage (Figure 4) were also associated with more severe forms of pulp inflammation. The presence of bacterial microleakage appeared to increase the severity of pulp inflammation, in comparison with non-infected cavity restorations, for post-operative periods between one and 54 weeks (Figure 5). In the absence of bacteria, pulp inflammation with all restorative materials was found to be absent or moderate, whereas in the presence of bacteria,
pulp inflammation was moderate or severe becoming necrotic (Figure 5). The greatest levels of inflammation were observed 7 to 14 weeks after surgery, thereafter the severity of pulp inflammation was observed to decline (Figure 4). These findings illustrate the importance of placing restorative materials, which prevent bacterial microleakage, in order to avoid pulp inflammation and post-operative complications.

The complexity of the interactions between variables mediating pulp injury and repair activity is such that the present review is not exhaustive, however, it has identified some of the key variables involved. Patient factors, such as age, diet, health, previous treatment history and allergies, should also be considered in relation to pulp responses (Figure 6), although information is more limited on these effects. Perhaps the most completely studied is that of ageing, where compromised pulp vitality and reductions in pulp repair activity have been observed. It is important to investigate and understand all of these pulp dentine changes, as well as their chemical action.

1. Use adequate coolant during cavity preparation, avoid unnecessary dentine removal and maximise the cavity RDT.
2. Select appropriate materials for the restoration being undertaken, using them to enhance pulpal protection by reactionary dentine secretion beneath cavities with small RDTs. Consider the biological effects of cavity etchants as well as their chemical action.
3. Avoidance of bacterial microleakage will encourage restoration longevity and reduce post-operative complications. The presence of bacteria will increase the severity and duration of inflammation in the pulp, thereby contributing to such complications.
4. Placement of materials which prevent bacterial microleakage will avoid inflammation and optimise treatment outcomes. Use of adhesive bonded materials is particularly technique sensitive if a good seal with the cavity wall is to be achieved.
5. Treatment planning based on harnessing the natural regenerative properties of the dentine-pulp complex should provide optimal clinical outcomes.

References