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The Effect of Holmium Laser Radiation on Stress, Temperature and Structure of Cartilage

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Abstract. It is difficult to permanently alter the shape of cartilage in a controlled way. In otolaryngology and plastic surgery several procedures are done to alter the shape of cartilage, for example the correction of a deviated nasal septum and surgery for bat ears. The aim of this paper is to study the main parameters which are necessary for the phenomenon of reshaping of cartilage under non-destructive laser radiation. We have measured temperature and stress in cartilage when it is being reshaped with a holmium laser, It has been shown that laser-induced stress relaxation in cartilage takes place when the tissue temperature exceeds 70°C. We have determined the conditions which allow the shape of cartilage to be altered without producing any pronounced alteration to matrix structure or chondrocytes.

Keywords: Cartilage; Chondrocyte; Laser; Stress relaxation; Structure alterations; Thermal and mechanical effects

INTRODUCTION

The phenomenon of stress relaxation and reshaping of cartilage under laser radiation has been described previously [1–5]. We have shown that the laser can shape cartilage to a new stable configuration of cartilage and this mechanism is due to a bound-to-free phase transition of cartilaginous water, taking place at about 70°C. Thermal, optical and mechanical effects accompany this process [6]. Alterations in the tissue structure during laser shaping of cartilage have been studied [4,7–10]. In attempting to reshape cartilage of about 1 mm in thickness, the use of a CO_2 laser leads to overheating of the surface, which occurs at approximately 130°C, with consequent damage to the cartilage [5,8]. In comparison to CO_2 lasers, a holmium laser allows thicker cartilage to be treated without overheating the surface and it also avoids any fundamental change in the cartilage matrix [7]. In order to prevent matrix damage it is necessary to regulate laser parameters of exposure time, rate and laser intensity [11]. Although these studies have helped to determine the nature of stress relaxation and reshaping of cartilage, little is known about its effect on chondrocytes. The short delivery time of laser energy minimises thermal damage to cartilage proteins [5] and therefore it is possible to optimise the delivery of laser energy to minimise damage to biological tissues in comparison to other methods.

In this paper we study the effect of holmium laser radiation on stress relaxation, chondrocytes and matrix structure. We measured stress and temperature during laser treatment and define the ‘gap’ which allows laser shaping, but avoids damage to cartilage. The aim is to establish what are the optimal conditions for laser shaping of cartilage.

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MATERIALS AND METHODS

Samples of human cartilage were collected from patients during intranasal procedures on the nasal septum, where the section of cartilage would have otherwise have been discarded. Samples of cartilage were cut into sections 1.2 mm in thickness and 12 mm in length. Laser shaping was performed as was described previously [4,6,7]. A holmium laser was used with wavelength of 2.1 μm, pulse energy of 0.5 J and duration of 0.5 ms, repetition rate of 5 Hz. Spot diameter (d) was measured by the conventional beam profiling method and varied from 4.0 to 7.8 mm by changing the distance between the fibre tip and the cartilage surface. These parameters allowed laser fluence (F) to be controlled from 0.85 to 3.2 J/cm. The irradiation time (t) for each spot was 4, 9 or 20 s. The effect of laser radiation on mechanical stress was examined with a tensometer which measured the force made by the deformed cartilage without any movement of the sample. The tissue temperature was detected with a needle-shaped thermocouple of 30 µm in diameter inserted 0.5 ± 1 mm into a small incision made by a scalpel with marking in the posterior side of the cartilage. Signals from the tensometer and from the thermocouple were visualised with an oscilloscope.

The structural changes to the matrix were examined and has been described elsewhere [4,6–10]. Longitudinal sections of cartilage were fixed in glutaraldehyde, embedded in epoxy resin and strained with toluidine blue. Sections were examined with a light microscope and particular attention was given to the appearance of the proteoglycans and chondrocytes. Two types of cell alteration were distinguished: cytoplasmic focal vacuolation (FV) which may be associated with reversible cell injury, and nuclear condensation (NC) which is generally regarded as being indicative of cell death. The histological changes in chondrocytes were quantified using a semi-quantitative rating of severity according to the proportion of cells affected. Severity was rated from none 0, minor +, moderate + + and severe + + +.

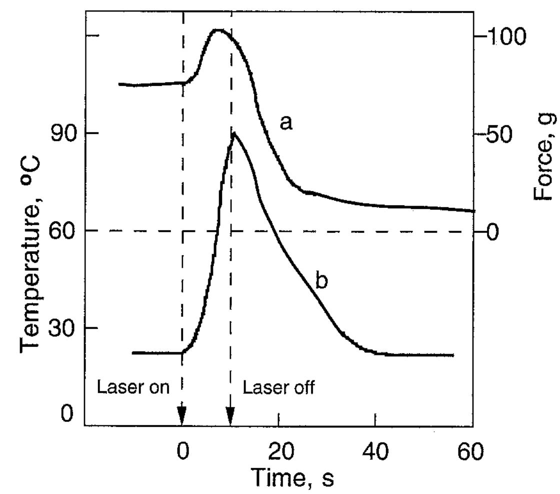

Fig. 1. Time dependency of (a) stress and (b) temperature in irradiated cartilage with F=1.7 J/cm^2, t=9 s.

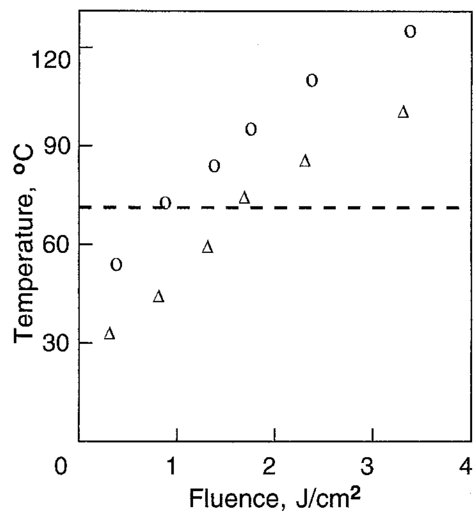

Fig. 2. Cartilage temperature as a function of laser fluence for various exposure times (△) t=4 s (○) t=9 s. The dashed line represents the boundary for the conditions needed for laser shaping.

RESULTS

The relationship between time, stress and temperature in cartilage are shown in Fig. 1. Stress increases during the first few seconds, then drops over 10–20 s. The temperature rises during irradiation and decreases when the laser is switched off. Figure 2 shows the almost linear dependency of cartilage temperature on laser fluence for 4 and 9 s of treatment. Deviation from a linear temperature relationship is due to the spatial spreading of heat. This is more important for laser spots with a small diameter. Laser shaping of cartilage took place with all regimes when the temperature exceeded 70°C.

The results of the histological analysis (Table 1) show how the changes in chondrocytes relate to the parameters of laser treatment (beam diameter, tip distance and treatment time), to the temperature recorded from a thermocouple, and whether laser shaping of cartilage was achieved. Examples of the histological appearance of cartilage undergoing shaping with a holmium laser are shown in Fig. 3. In addition to various types of structural alterations described elsewhere [4,7–11], there is also chondrocyte vacuolation (Fig. 3a) and nuclear condensation (Fig. 3b). The conditions for cartilage reshaping which produced no pronounced changes in the matrix structure at light microscopy are shown in Fig. 4. The 'gap' between the conditions needed for laser shaping and those which produce structural

Table 1. Chondrocyte changes in cartilage following holmium laser radiation

Tip–sample distance (mm)	Effective laser beam diameter (mm)	Laser beam fluence (J/cm^2)	Treatment time (s)		
			4	9	20
17	4.0	3.2	*(95–100°C) FV+ NC+++	(135–140°C) FV++ NC+++	No test
20	4.7	2.3	*(85°C) FV+ NC++	*(105–125°C) FV++ NC+	(190–200°C) FV+++ NC+++
23	5.4	1.7	*(75–80°C) FV+ NC0	*905–95°C) FV+ NC+	No test
28	6.6	1.2	(50–55°C) FV0 NC+	*(85–90°C) FV+ NC+	No test
34	7.8	0.85	(40–45°C) FV0 NC0	*(70–75°C) FV0 NC++	No test

*Indicates that laser shaping occurred.
FV, Focal vacuolation; NC, nuclear condensation.
Proportion of chondrocytes affected=minor +, moderate ++ and severe +++.

changes in the cartilage matrix decreases with treatment time. Damage to chondrocytes was minimised with laser fluence of 1.7 J/cm for 4 s (Table 1). Laser heating with parameters less than this produce minimal cell damage, but no laser shaping. If the laser fluence was increased or if the treatment time was prolonged, significant nuclear condensation occurred. Histopathological analysis also showed that the amount of chondrocyte damage varied throughout the thickness of the cartilage as the more superficial cells showed morphological evidence of damage whilst many of the cells in the deeper main body of the cartilage remained undamaged (Fig. 3c).

DISCUSSION

We found that laser-induced stress relaxation in cartilage is based on temperature kinetics. At the beginning of laser treatment the increase in stress may be due to tissue expansion under heating when water does not have enough time to move the periphery of the laser spot (water inertial effect). After a few seconds, the movement of water leads to a redistribution of stress and results in stress relaxation. This process is controlled by the kinetics of heating, and in particular by the duration of the period when cartilage temperature exceeds 70°C. As was shown previously [4,7–11], a temperature of 70°C is needed in order for a phase transition of bound-to-free water to occur in the cartilaginous matrix. The temperature within the treated tissue increases with laser fluence and with exposure time. If the temperature is higher than 70°C phase transition is faster, but the heat may damage chondrocytes or/and matrix. The main parameters affecting laser energy which include laser fluence and exposure time, have to be considered in order to find the right conditions for laser shaping of cartilage.

In comparison to the results of histological analysis of cartilage reshaped by laser radiation with wavelengths 10.6 μm [3,8] or 1.44 nm [9,10], when considerable alterations in cartilage matrix were found, our results define the 'gap' which allows laser shaping, but avoids damage to cartilage matrix. We have shown, in vitro, that a laser treatment time of 4 s can be enough to allow stress relaxation to occur, but with tip–sample distances of more than 28 mm energy delivery is inadequate to cause an alteration in cartilage structure. A treatment time of 9 s produces laser shaping, but it is

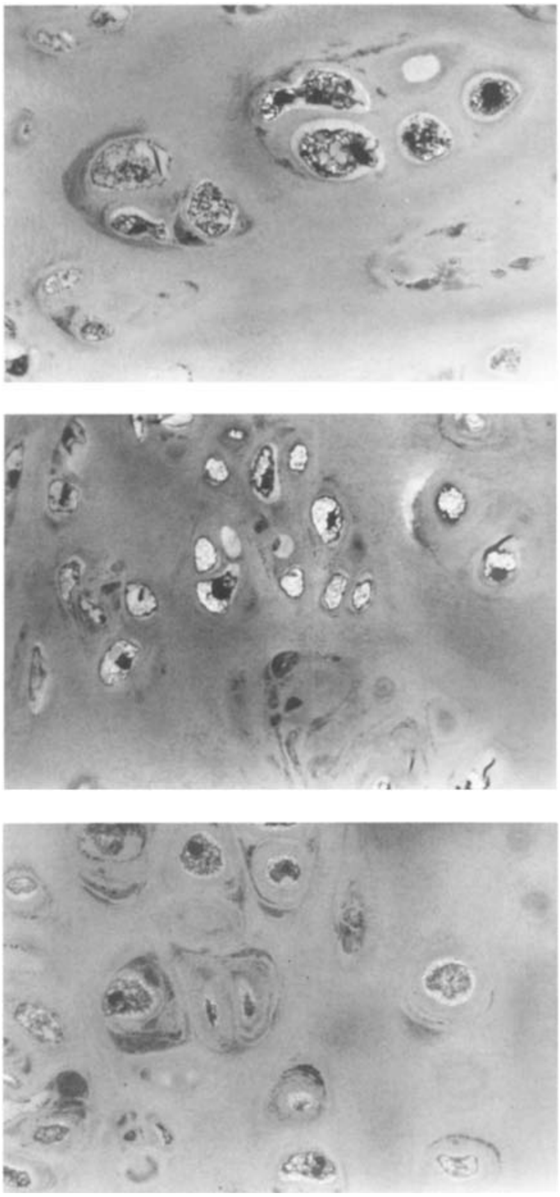

Fig. 3. Structure of irradiated cartilage (diameter of field 110 μm). (a) F=2.3 J/cm^2, t=9 s, slight visible changes in matrix, minor nuclear condensation, and moderate focal vacuolation; (b) F=3.2 J/cm^2, t=9 s, dramatic visible changes in matrix, severe cells with nuclear condensation, and moderate focal vacuolation; (c) F=1.7 J/cm^2, t=4 s, no visible changes in matrix, no nuclear condensation, some minor focal vacuolation.

accompanied by pronounced changes in the structure. This can easily be understood as the denaturation of proteoglycans takes more time than that required for stress relaxation which occurs as a result of a reorganisation and short-range movement of water molecules. The use of a different type of laser or regimes may alter the 'gap' at which stress relaxation occurs and at the same time avoid producing tissue damage.

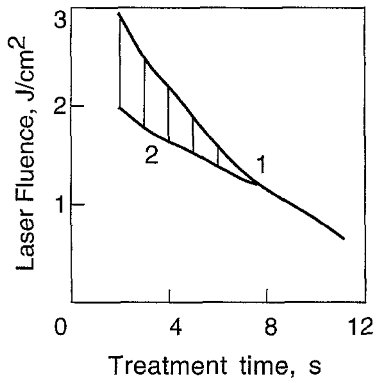

Fig. 4. Optimal condition for laser shaping of cartilage with a holmium laser (dashed). 1, Boundary of changes of matrix structure; 2, boundary of laser shaping.

Chondrocytes are more sensitive to damage with laser treatment than the cartilage matrix. In our experiments, laser shaping was always accompanied by some alterations in chondrocyte morphology. However, there are conditions (t=4 s, F=1.7 J/cm^2) which allow laser shaping without nuclear condensation and only produce minor cell vacuolation. The effect of cytoplasmic vacuolation alone on the long-term integrity of the cartilage is not known and an in vivo study is planned to examine this. The optical properties of cartilage in vitro differ from those of living tissue and also depend on the age and type of cartilage. Therefore, the optimal condition for laser treatment shown in Fig. 4 will be investigated during future in vivo studies.

CONCLUSIONS

1. Laser-induced stress relaxation in cartilage takes place when the tissue temperature exceeds 70°C and is in temporal accordance with the temperature kinetics.
2. Laser energy density and exposure time are the main parameters which determine the laser shaping of cartilage.
3. It is possible to create the conditions for laser shaping with few changes in cartilage matrix.
4. Chondrocytes are more sensitive to laser treatment than the cartilage matrix. We found conditions which produce cartilage shaping with the holmium laser, but which only cause minor cell vacuolation without nuclear condensation.

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