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Fracture and fatigue in osteocytes

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ABSTRACT

Fatigue is a common mode of mechanical failure which occurs when a material is subjected to repeated cycles at a strain level less than that needed for monotonic fracture. Fatigue has been observed and measured in many different materials but, until recently, not in cells. We devised a novel experiment which allowed us to create both monotonic failure and fatigue in the cellular processes of osteocytes within samples of bone (Dooley et al., European Cells and Materials 2014). In the present paper, we describe the results of further experiments and a computer simulation, which has allowed us to estimate the strain history of each sample tested and thus present, for the first time, strain/life data for cells. Failure occurred during the first cycle at strains of 0.1–0.2; at lower strains failure occurred after a number of cycles which depended inversely on the applied strain range. Scatter in the strain/life data was reduced when we allowed for the effects of mean stress using the Smith–Watson–Topper parameter. We confirmed that aspects of our experimental method (the types of microcrack used and the testing of fresh versus frozen samples) did not affect the results. Such information is useful because many cell types, including the cellular processes of osteocytes, experience cyclic strain in vivo.

1. Introduction

Engineers and materials scientists are very familiar with the term "fatigue", which refers to the gradual failure of a material when it is subjected to cycles of strain, the largest value of which is not sufficient to cause failure if applied only once in so-called "monotonic" loading. In medical circles the word fatigue has a different meaning, and the failure of a tissue such as bone under cyclic loading is referred to as a "stress fracture". Fatigue failures are divided into two groups – "low cycle fatigue" and "high cycle fatigue" – according to the number of cycles to failure. Low cycle fatigue – the subject of the present paper – occurs when a material is loaded to a maximum strain which, in a monotonic test, would cause non-reversible behaviour such as plastic strain, microdamage or viscoelasticity over a significant proportion of the material volume. Damage accumulates quickly on each cycle leading to failure in a relatively small number of cycles. Fig. 1 shows typical test data (Boller and Seeger, 1987), in which the number of cycles to failure N_f is plotted as a function of the strain range $\Delta\varepsilon$, defined as the difference between the maximum and minimum strain in the cycle. Data are usually

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presented on a logarithmic plot and often fall approximately on a straight line, giving the following empirical relationship in which C and n are constants:

$$N_f = \frac{C}{\Delta\varepsilon^n} \quad (1)$$

It has been demonstrated that fatigue occurs in many different types of materials, including metals, ceramics, polymers and composites (Stephens and Fuchs, 2001; Hertzberg and Manson, 1980), and also in natural composite materials such as bone (Taylor, 1998) and wood (Salmi et al., 2012), but until very recently fatigue had not been detected in mammalian cells. Indeed, there are almost no published data on any kind of mechanical failure mode for cells. Exceptionally, the failure strain of the outer cell membrane (which consists of a bilayer of lipids) was measured at 0.02–0.03 (Nichol and Hutter, 1996). Failure of this membrane may not lead to total rupture of the cell, because the cell is also supported by a cytoskeleton and may be able to repair local membrane damage (Sheetz et al., 2006). Pipette aspiration has been used to cause membrane rupture in red blood cells and thus estimate membrane strength (Rand, 1964).

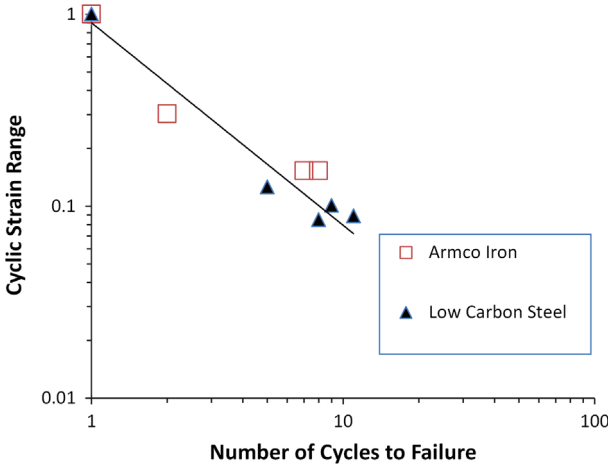

Fig. 1 – Typical data from low-cycle fatigue testing, in this case for two metallic materials (Boller and Seeger, 1987). The number of cycles to failure is plotted logarithmically against the applied strain range.

In summary, there is a lack of data in the literature reporting how much strain is needed to permanently rupture a cell, whether loaded monotonically or cyclically. This information is important because many types of cells experience cyclic strain. For example endothelial cells undergo strains of the order of 0.22 (Mofrad and Kamm, 2010) as a result of fluid shear. Cyclic strain levels of the order of 0.05 are necessary for the differentiation of various cell types (Mofrad and Kamm, 2010); the osteocyte network is ruptured in the vicinity of microdamaged bone (Colopy et al., 2004) and this rupture takes the form of fatigue failures of individual cellular processes (Dooley et al., 2014).

Recently we devised an experiment which allowed us to carry out this type of testing on bone cells (osteocytes). Our method (Dooley et al., 2014) takes advantage of the fact that osteocytes live inside the bone matrix and are connected to each other via long, thin extensions of the cell body known as cellular processes (Fig. 2). We noticed that if there is a crack in the bone matrix, these processes can be seen passing across between the crack faces (Fig. 3). By applying forces to a sample of bone we were able to cause a crack to open and close, thus applying strain to the processes spanning it. Some processes failed immediately on the first loading cycle, whilst others failed after a number of cycles.

We thus demonstrated the existence of fatigue failure in cellular processes, which are made from the same constituents as other parts of the cell membrane and cytoskeleton. However this experiment had some limitations. We were not able to quantify the applied strain because, though we could measure the opening displacement of the crack, we did not know the total length of the process, and processes are known to adhere to the bone matrix via focal adhesion points (McNamara et al., 2009), which will affect the distribution of strain along their lengths. We also had some concerns about some aspects of our methods for sample preparation.

The work described in the present paper aimed to answer the following questions:

(1) What are the strain levels at which monotonic rupture and low cycle fatigue failure occur? To answer this

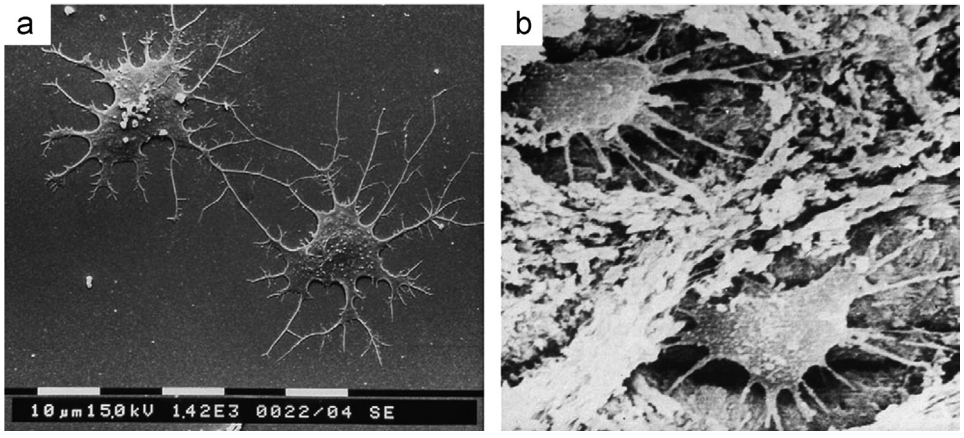

Fig. 2 – Osteocytes are linked together in a network via numerous cellular processes: these images show examples of cells without (a) and with (b) the surrounding bone matrix (Klein-Nulend et al., 2005).