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Accurate *in vitro* identification of fracture onset in bones: Failure mechanism of the proximal human femur

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ABSTRACT

Bone fractures have extensively been investigated, especially for the proximal femur. While failure load can easily be recorded, and the fracture surface is readily accessible, identification of the point of fracture initiation is difficult. Accurate location of fracture initiation is extremely important to understand the multi-scale determinants of bone fracture. In this study, a recently developed technique based on electro-conductive lines was applied to the proximal femoral metaphysis to elucidate the fracture mechanism. Eight cadaveric femurs were prepared with 15–20 electro-conductive lines (crack-grid) covering the proximal region. The crack-grid was connected to a dedicated data-logger that monitored electrical continuity of each line at 700 kHz. High-speed videos (12,000 frames/s, 0.1–0.2 mm pixel size) of the destructive tests were acquired. Most crack-grid-lines failed in a time-span of 0.08–0.50 ms, which was comparable to that identified in the high-speed videos, and consistent with previous video recordings. However, on all specimens 1–3 crack-grid-lines failed significantly earlier (2–200 ms) than the majority of the crack-grid-lines. The first crack-grid-line to fail was always the closest one to the point of fracture initiation identified in the high-speed videos (superior–lateral neck region). Then the crack propagated simultaneously, at comparable velocity on the anterior and posterior sides of the neck. Such a failure pattern has never been observed before, as spatial resolution of the high-speed videos prevented from observing the initial opening of a crack. This mechanism (fracture onset, time-lag, followed by catastrophic failure) can be explained with a transfer of load to the internal trabecular structure caused by the initial fracture of the thin cortical shell. This study proves the suitability of the crack-grid method to investigate bone fractures associated to tensile stress. The crack-grid method enables significantly faster sampling than high-speed cameras. The present findings elucidate some aspects of the failure mechanism of the proximal human femoral metaphysis.

1. Introduction

Bone fractures represent a severe clinical condition. In addition to those consequent to high-energy trauma, there is a growing number of fragility fractures in the elderly population, often regarded as a pandemic disease (Kanis et al., 2007) for which current risk assessment methods are inadequate (Wilkin and Devendra, 2001). Bone fracture is a complex process, and is inherently multiscale in space and time (Viceconti et al., 2012). At physiological loading-rates, the macroscopic failure of a bone segment occurs abruptly through the disruptive propagation of microscopic cracks (Hansen et al., 2008). Bone fracture is determined by a multiscale-combination of anatomy, and local bone tissue properties. Such variables depend on a number of factors, including age, bone quality, diseases (including osteoporosis). Identifying the spot where fracture initiates (i.e. the weakest point a bony structure) would be extremely useful to elucidate the mechanism of fracture in healthy and diseased bone.

So far, a number of tests have been conducted at the macro-scale, measuring the ultimate load of whole bones, and replicating clinically relevant fracture scenarios (Cristofolini et al., 2007; Juszczyk et al., 2011). Therefore, such tests are the gold standard for the validation of models aimed at predicting bone fracture (Bessho et al., 2007; Schileo et al., 2007). However, as the entire fracture process can take shorter than a millisecond (Juszczyk et al., 2011), a major limitation of such *in vitro* tests is the difficulty in identifying the point of fracture onset, which is key information for model validation. This limitation was partly overcome using high-speed videos (Cristofolini et al., 2007; Schileo et al., 2008; de Bakker et al., 2009; Cristofolini et al., 2011). However, sometimes frame-rates as high as 15,000 fps are not sufficient to identify the point of fracture onset, and a trade-off between frame-rate and spatial resolution is needed. Furthermore, cost of suitable high-speed cameras is high.

Other techniques exist to detect propagating cracks in different fields. Acoustic emissions (AE) were applied to bone (Guyer and Dauskardt, 2004; Van Toen et al., 2012). However, identification of

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the point of fracture initiation based on two AE-detectors is intrinsically impossible when the structure is inhomogeneous and anisotropic (Hatakeyama et al., 2000; Reilly et al., 2005). Adhesive sensors such as grid-based crack-propagation (Miles and Tanner, 1992), or foil crack gauges made of constantan sheet (Krak-Gauge, Rumul, Neuhausen, CH) or a graphite layer (Hatakeyama et al., 2000) can be applied only on flat or single-curvature surfaces. The deposition of electro-conductive lines on the surface of a specimen (Chang, 1989; Moore et al., 2001) may be an alternative. It was once applied to bone, but on flat specimens (Vashishth et al., 1997). Only recently, a method has been reported that enables application of a series of electro-conductive lines (Crack-Grid, CG) on any surface (including double-curvature) (Juszczyk et al., 2010), and determination of fracture through a data-logging system (Break'O'Meter, BOM) that samples each CG-line at 700 kHz. In a preliminary demonstration, a spatial resolution of 0.5 to 2.0 mm was tested. This method can detect the actual sequence the lines fail due to tensile strain, thus it should well fit the testing of complex structures (such as bones) where the point of fracture initiation and the propagation are under investigation.

This paper will focus on the characterization of the disruptive phase of crack propagation in bones. In fact, predictive numerical models of bone at the organ-level seem to be the most promising approach to reliably assess skeletal fracture risk (Crawford et al., 2003; Schileo et al., 2008) and evaluate preventive therapies (Cody et al., 2000; Keaveny et al., 2008). To develop and validate such models, a thorough characterization of the bone fracture process is necessary. Aims of the present study were:

- To assess if the newly proposed method based on a Crack-Grid for identifying the point of fracture initiation (Juszczyk et al., 2010) can be applied to a real bone such as the femur. Accuracy of the proposed method was assessed by comparison against high-speed movies of the same fracture event, which were taken as a golden standard (Cristofolini et al., 2007).
- To use the Crack-Grid to identify the point of fracture initiation, and the fracture mechanism in the proximal human femoral metaphysis during *in vitro* simulated spontaneous fracture.

2. Materials

In order to detect initiation of bone fracture, the relevant region of the bone surface was covered with a series of parallel lines of electro-conductive dye (ECD) forming a Crack-Grid (CG). Such CG-lines must intersect the plane where fracture is expected to occur (however, they do not necessarily need to be perpendicular to the plane of fracture). Disruption of a CG-line indicates that the bone surface was locally fractured. Each line was connected to a custom-designed data-logger capable of detecting loss of electrical conductivity. Accurate identification of the time of disruption of each line enabled detecting the point of fracture initiation.

2.1. Bone specimens

A total of 8 cadaveric fresh-frozen human femurs (Table 1) were obtained through ethically-approved donation programs. They were DEXA-scanned (Excel-Plus, Norland, USA) and CT-scanned (HiSpeed, General-Electric, USA) to document bone quality and lack of abnormality or defects. Anatomical dimensions (head diameter and biomechanical length) were measured (Table 1 (Ruff and Hayes, 1983; Cristofolini, 2012)). To preserve hydration, during preparation and testing the femurs were wrapped in soaked cloths and sprayed with saline solution during testing.

2.2. Crack-grid application

The crack-grid preparation is derived from a validated procedure for bonding strain gauges to bone (Viceconti et al., 1992; Bessho et al., 2007; Cristofolini et al., 2009; Yosibash et al., 2010), and is not known to alter bone tissue properties. Preliminarily, the bone surface was cleaned of all soft tissues, degreased with ethanol, and a cocktail of acetone and 2-propanol (RMS1, HBM, Darmstadt, Germany), and spread with ethyl-cyanoacrylate glue (Super-Attak Easy-Brush, Henkel-Loctite, Zingonia, Italy) to provide a brittle electrically insulating support, and enhance adhesion of the ECD dye. A mask was used to prepare each specimen with 15–20 CG-lines (actual number and spacing depended on the specimen's anatomy), corresponding to a resolution of 2–4 mm in the relevant region (Fig. 1). The CG-lines were directed along the neck axis (i.e. perpendicular to the expected fracture (Rockwood et al., 1991)). The CG-lines were deposed by spraying an ECD based on butyl-acrylate and copper (EMV35, CRC Industries BVBA, Zale, Belgium).

In a previous methodological study (Juszczyk et al., 2010), it was found that: (i) such CG-lines correctly adhere to the bone; (ii) the CG-lines can withstand a strain exceeding 20,000 microstrain (larger than failure strain of bone(Bayraktar et al., 2004)) without electric failure; (iii) failure of the CG-line occurs abruptly when a gap of less than 1 μm is created in the underlying material; (iv) the method is suitable to double-curvature surfaces such as the proximal femur.

2.3. Data logging

To record the time of disruption of each CG-line, a dedicated data logger (Break'O'Meter, BOM) was used (Juszczyk et al., 2010) based on a self-contained board (MB-128-MAX, MikloBit, Jaworzno, Poland) and a 16 MHz microcontroller (Atmega-128, Atmel-Corporation, San Jose, USA). The BOM was capable of sampling up to 32 CG-lines at a frequency of 700 kHz.

2.4. Loading conditions

The femurs were tested to failure following a validated protocol (Cristofolini et al., 2007), which enabled replicating *in vitro* the spontaneous fractures of the proximal metaphysis. Spontaneous fractures derive from physiological or para-physiological loading (e.g. sudden muscle contraction due to stumbling or mis-stepping), but not from a traumatic event (Jeffery, 1974; Rockwood et al., 1991). This type of fracture has received great interest recently because of its intriguing mechanism, which includes poor bone quality and compromised neuro-motor control (Juszczyk et al., 2011; Viceconti et al., 2012). Fracture is caused by a hip joint force, which is nearly aligned with the femoral axis, and is primarily associated with tensile strain on the superior–lateral part of the neck (Keyak, 2000; Gomez-Benito et al., 2005; Bessho et al., 2007;

Table 1
Details of the specimens. In the first columns, details of the donors are listed. Bone quality is reported in the 6th and 7th column (*T*-score of the bone density referred to the young reference population, and *Z*-score referred to the age-matched population, based on the Norland DEXA scanner reference population). Biomechanical dimensions (Ruff and Hayes, 1983; Cristofolini, 2012) are reported in the 8th and 9th columns. In the last column, the failure load is reported.

Femur♯	Details of the donors				Details of the femurs					Failure load
	Gender	Age at death	Donor Height (cm)	Donor Weight (kg)	Side	DEXA T-score	DEXA Z-score	Head diameter (mm)	Biomech length (mm)	(*N*)
1	Female	n.a.	n.a.	n.a.	Right	−4.54	−2.87	47.0	438	3017
2	Female	n.a.	n.a.	n.a.	Right	−2.05	−0.38	46.0	427	5337
3	Male	n.a.	n.a.	n.a.	Right	n.a.	n.a.	51.0	472	2227
4	Female	49	170	82	Right	−0.33	0.38	42.0	443	10,860
5	Male	65	188	95	Right	−0.51	0.66	52.0	476	12,350
6	Male	62	188	74	Right	−2.14	−1.15	54.5	464	8652
7	Male	62	188	74	Left	−2.58	−1.32	55.0	463	6382
8	Male	79	191	61	Right	n.a.	n.a.	54.0	471	10,860