INTRODUCTION
Joint impact trauma is frequently sustained through accidents which involve falls from a height or direct blows to the joint. Despite careful early surgical reconstruction, the development of post-traumatic osteoarthritis is frequently a late consequence of joint impact trauma and may lead to disability and subsequent surgical intervention (i.e. joint arthroplasty, replacement). Links between single traumatic events and osteoarthritis have previously been reported for various clinical cases. However, the cellular pathways underlying progressive cartilage destruction remain largely unknown. Previous studies focused on cartilage damage at load levels sufficient to fracture the underlying bone or lead to macroscopic fissures and fissuration of the cartilage layer. It was the goal of this in vitro investigation to determine and quantitatively analyze which impact trauma magnitude causes the immediate cellular respectively structural damage to the cartilage layer. Understanding the initial cellular damage may help to find the missing link between the traumatic impact and structural damage of cartilage layers in post-traumatic joint diseases such as trauma-induced osteoarthritis.

METHODS
12 fresh porcine patellae were harvested from 6 Yucatan minipigs (12-14 m) within 1 h of sacrifice and placed in bovine serum at 37 °C to conserve viability. During processing, the cartilage was kept moist. Each patellar surface was divided into quadrants. Using a drop tower device, a mass was dropped from various heights resulting in different impact energies (0.06, 0.1 and 0.2) onto one of three quadrants while the remaining quadrants served as control. Impact force and cartilage deformation were recorded during testing to verify reproducibility. 4 patellae were stained with India ink to identify surface disruptions, fixed, freeze fractured and prepared for electron microscopy. From the remaining patellae, blocks of full-thickness cartilage were removed from each quadrant. Each block was stained with propidium iodide (PI) as well as with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). 10 μm cryo-sections were taken from each specimen and photographed within 2 hours (light microscope, 400x). Each cartilage section was divided into a deep, intermediate and superficial zone. Functional and non-functional cells were counted by two independent observers. In the MTT staining, death rate was expressed as a ratio of dead cells to total cell population in each section with the PI staining serving as control. A Wilcoxon test was chosen for statistical evaluation.

RESULTS
All impact energies produced a transient indentation on the articular surface, without disruption or fissures of the surface (India ink). Electron microscopy unveiled no discernable evidence of structural damage in all specimens and layers (Fig. 1). Equally intense staining signals under normal as well as fluorescent light allowed to distinguish clearly between functional (MTT) and non-functional cells (PI). Significant differences among the death rates for different impact energies were seen for all specimens: Increasing impact energies led to an increasing number of non-functional cells compared to the control (Fig. 2). Statistical analysis revealed a significantly increased death rate for the superficial and intermediate cartilage layer compared to the deep one at larger energy levels (energy > 0.1 J, Fig. 2).

DISCUSSION
In this study, impact trauma magnitudes were identified which led to immediate cellular damage of the cartilage layer. The results suggest that prior to structural destruction considerable damage is experienced at the cellular level even at low energy trauma. These initial trauma-induced cellular dysfunction may indeed act as a precondition for ongoing tissue damage. This is of particular importance since sufficient synthesis of cartilage matrix proteins is directly dependent on tissue viability.

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REFERENCES