INTRODUCTION:
Extracorporeal shock wave therapy (ESWT) is nowadays applied to a variety of bone and soft tissue pathologies in orthopedics [1]. Compared to the commonly used focused shock waves, radial ESWT (rESWT) is characterized by larger treatment area, which simplifies application and reflects pathology zone rather than a point. Therefore, rESWT is expected to be at least as effective as the focused ESWT. rESWT has been proven efficient in enthesiopathies, but is considered critically with bone pathologies due to its’ lower energy level. The purpose of the study was to evaluate if rESWT can induce new bone formation at low energy flux densities and to study the time course of rESWT-induced osteogenesis.

METHODS:
New Zealand white rabbits (n=13) were used for the animal model after approval by the responsible ethics committee. After the adaptation phase, radial extracorporeal shock waves (rESW) were applied with the Swiss Dolorclast shock wave device (EMS Electro Medical Systems, Nyon, Switzerland) to one randomized femur of each animal, while the contralateral side served as an intraindividual control. The application site was localized at the ventral thigh precisely superior to the patella with the rabbit in supine position. 4000 pulses of rESW with an energy flux density of 0.16mJ/mm² were applied twice with standard parameters (8Hz, 4 bar, 7 days interval).

Intravital staining for new bone formation was performed with fluorescent dyes administered subcutaneously: a) tetracyclin (25mg/kg, day 4-1 before rESWT), b) calcein green (20mg/kg, day 4-6), c) alizarin red (30mg/kg, day 18-20) and d) calcein blue (30mg/kg, day 31-34 after both rESWTs).

Animals were sacrificed at 1 week (n=4), 3 weeks (n=4) and 5 weeks (n=5) after the second rESWT. Rabbit femurs with adjacent soft tissues were removed and fixation was performed in methanol and PMMA. Sections of all femora (thickness ~75µm) were investigated with broad-band fluorescence microscopy (H3 filter, JUST filter) and contact microradiography for new periosteal and endosteal bone and callus formation, periosteal detachment and cortical and trabecular fractures. New bone formation was evaluated by two blinded reviewers and classified according to Maier et al. [2]:
- class 1: no signs of new bone formation
- class 2: new endosteal and/or periosteal bone formation without covering the whole bone surface
- class 3: new endosteal and periosteal bone formation covering the whole bone surface

Statistical analysis of new bone formation in treated and untreated femora was performed with the exact Fisher-test with a P-value <0.05 considered significant.

RESULTS:
Integration of the fluorescent dyes into bands of newly deposited bone could be shown by fluorescence microscopy and was significantly increased after rESWT (fig. 1). Shock wave-induced osteogenesis was already visible at week 1, however, new bone formation was even more pronounced and significantly different to the control group after 3 and 5 weeks (fig. 2). The different colored fluorescent dyes allowed a description of the time flow of bone formation (fig. 1). Interestingly, induction of bone formation by rESWT could be demonstrated already in the first week after shock wave application and persisted for at least 5 weeks, which was found by the newly formed fluorescent bands with dyes applied in the late phase of the experiment (alizarin red and calcein blue). Furthermore we could demonstrate both endosteal and periosteal new bone formation at the dorsal femoral cortex after rESWT, but not in the control.

REFERENCES:

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