European Cells and Materials Vol. 5. Suppl. 2, 2003 (pages 51-52) ISSN 1473-2262

FRACTURE HEALING IN VITRO

M.Ahrens [1,2], U.Schlegel [1], H.Halm[2]

[1] AO Research Institute, Davos, CH

[2] Clinic for Spine Surgery, Academic Teaching Hospital (Univ. Luebeck) Neustadt, D

INTRODUCTION: Since the early works of Fell in 1929 [1] it is known that femoral rudiments, isolated from newborn fowls are capable of undergoing considerable histological and morphological development when cultivated in vitro. Other researchers from those early days of tissue culturing already used Fells model for studying fracture repair *in vitro* [3, 4] with loosely attached fractured bone ends. However, a current literature search revealed only a few publications using isolated bones in respect to fracture healing [2]. Using modern cell culture technologies (sterile laminar flow, defined culture media, incubator) the following study was designed to elucidate the prospective of using isolated complete rat femurs as a model for studying fracture repair in vitro.

METHODS: Wistar rats aged 6 days were used in three groups, 10 animals each, with the endpoint of one, two or three weeks. Both femora of every animal were explanted under sterile conditions in a laminar flow with the use of microsurgical instruments and a stereomicroscope. All bones were intact macroscopically after explantation and stripping of the periosteum. The remaining tissue at the bone surface was mildly digested in growth media with 0,02% Collagenase A for 45 minutes, followed by washing in Tyrode's Balanced Salt Solution (TBSS). Custom-made plates (0.5 x 1.5 x 3 mm) from high-grade silicone (Angst und Pfister AG, Zurich, CH, Cat.No.AP1010192503) were steam sterilized and used as an external fixation frame. Four sterile acupuncture needles with a diameter of 0,3 mm (C-Type, No. 8, 0.30 x 50 mm SEIRIN, Germany) were inserted in a rectangular fashion through a gauge with four evenly spaced 0.3 mm openings into the bone and the silicone plate. The artificial fracture was made by a cut in the middle of the diaphysis with a scalpel on the surface of a sterile glass slide. The fixed bones were submersed in 6-well culture dishes with 5 ml media of BGJb (Biggers, Gwatkin, Judah with Fitton Jackson Modification; Life Technologies 22591-010) with 50µg/ ml sodium ascorbate. No antibiotics were used. Culture media was changed every 72 hours and inspected for infections. At the endpoint of every experiment the femurs were x-rayed with contact micro radiography, subsequently fixed in 4% buffered formalin and embedded in LR-White resin for further histological evaluation. The Movat pentachrome staining I was chosen to investigate the fracture gap and possible healing process.

RESULTS: Development of the rudiments in all groups continues to progress normally compared to uninjured, unfixed controls, i.e. increase in length and breadth, with normal histogenesis of cartilage and bone. During enlargement the epiphyses retained their characteristic shape, their histological differentiation continued and appeared similar to the in vivo pattern. The maximum increase in length during cultivation was about 30% after 3 weeks. No antibiotics were used, however there was only a dropout rate due to infection of 10% evenly distributed among the groups. After one week a fibrous tissue filled the fracture gap, which was partly replaced in the later course with osteoid, however the replacement by osteoid tissue in the epiphyseal regions continued normally. In cases of misalignment of the fracture gap larger than 0,5 mm calcification was only partly.

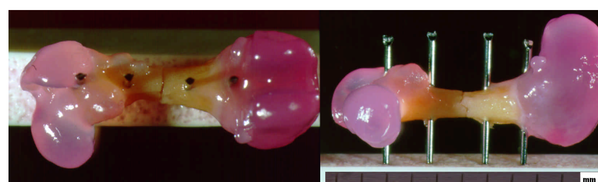

Fig. 1: View from above (left) and side view (right) of external fixed rat femur with four stainless steel acupuncture needles (0,3mm). Scale and white silicone frame below.

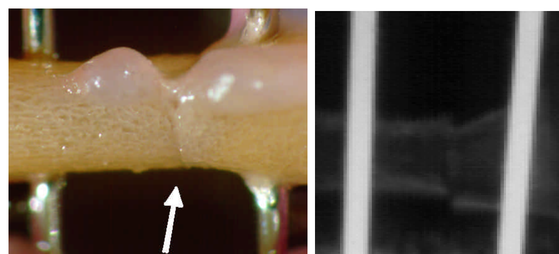

Fig. 2: Fractured femur, after 2 week in vitro culture, close up view (left), micro radiography (right).

DISCUSSION & CONCLUSIONS: The mammalian epiphysis is capable of continuing its characteristic histological differentiation even when deprived of nerve and blood supply and cultivated in a heterologous medium outside the animal body. In this experiments femora of newborn rats were used which still expresses embryological growth pattern but are on a higher stage of development compared to the rudiments used by Niven [4] and Krull [3], therefore external fixation was possible, resembling an osteosynthesis. The observed calcification of the fracture gap was also very dependent on the precise alignment of the fracture ends and was only partly in cases of misalignment larger than 0,5 mm. The author would like to acknowledge the authors of the listed references who described basic methods and ideas of our experiments 70 years ago, however only few studies were published ever since which made use of this old knowledge. Since the cultures are easy to handle and the setup is inexpensive, more sophisticated experiments involving growth factors could profit from this old, newly reinvented in vitro model.

REFERENCES: [1] H.B. Fell (1928) *Experiments on the differentiation in vitro of cartilage and bone. Part I.* Arch. F. exper. Zellf. 7: 390 [2] A.Y. Ketenjian, C. Arsenis (1975) *Fracture callus cartilage differentiation in vitro.* In Vitro 11(1): 35-40. [3] G. Krull (1937) *Untersuchungen ueber Frakturheilung in der Gewebekultur.* Arch Orthop Unfallchir 37: 131-137. [4] J.S.F. Niven (1931) *The repair in vitro of embryogenic bones after experimental fracture.* Arch exp Zellforsch 11:253-258.