

# Fracture healing and its potential as a bioreactor for skeletal tissue engineering

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## Background and Introduction:

Significant advances have been made in prosthetic replacement of components of the skeletal system using synthetic materials. Although the success of prosthetic replacement is good, the increasing demands both in terms of implant life with increasing longevity and level of activity still leave room for improvement. Long term aseptic loosening of prostheses, primarily attributed to wear debris related osteolysis results in a continuing need for revision surgery. The advances in biological tissue engineering, using cells and scaffolds *in vitro*, although promising, have not yet reached the levels of use seen with synthetic prostheses. Increased longevity is associated with an increase in degenerative disease of the skeletal system, particularly degenerative joint disease.

The skeleton can adapt to the ever changing mechanical demands throughout life, optimizing functional morphology through dynamic adaptation. However, accumulative fatigue damage or gross monotonic overload lead to gross fracture. It is well established that bone as both a tissue and structure has great capacity for repair and unlike many tissues can restore both microscopic and gross architecture under optimal fixation conditions. The mechanical environment at the fracture site is key in determination of both the pattern and progression of repair. The repair process is acutely sensitive to mechanical conditions particularly in the early stages. Conventionally, the repair process comprises three phases, inflammatory, reparative and remodeling. Indirect bone repair comprises a unique cascade of connective tissue differentiation from the fracture haematoma through the entire spectrum of connective tissues with the optimal outcome of regenerated lamella bone. Indirect bone repair also encompasses both intramembranous and endochondral ossification. There is also a suggestion from some studies of transdifferentiation during the repair process.

Lower order animals have the capacity to regenerate whole limbs after amputation, in mammals this regenerative capacity is lost,

however, the process of fracture repair demonstrates that regeneration of connective tissues can occur within the adult skeleton. Changing mechanical conditions at a fracture site may lead to delayed or non-osseous union, with fibrous or cartilaginous tissues remaining in the fracture gap. Modelling studies indicate that hydrostatic stresses lead to chondrogenesis and deviatorial strains to fibrous tissues. Specific stresses applied to bone in *in vivo* bone chamber experiments have also been shown to induce cartilage. Thus the repair process in bone can be manipulated by mechanical influences to modulate the tissues present as a function of time.

## Rational:

It may be hypothesized that specific hydrostatic stresses in the order of 0.5- 3MPa applied to a bone healing process will induce cartilaginous tissues. Furthermore, this process could be used in an *in vivo* bio-reactor to resurface joints with biologically engineered articular cartilage.

## Methods:

Three groups of six sheep received unilateral hip hemi-arthroplasties were sacrificed 24 weeks post-operatively to harvest the acetabula. At operation, acetabular cartilage was removed completely and the subchondral bone was reamed to bleed. Three femoral head sizes, 25-, 28-, and 32-mm, were used to induce different contact stress levels. In a fourth group three polyethylene pegs were inserted into the bone to support the femoral head and protect any regenerate tissue from load. Vertical ground reaction force (GRF) data were measured and normalised by body weight for both limbs pre-operatively and every 4 weeks post-operatively. Five Specimens each from the 25- and the 28-mm group and eight un-operated controls were processed and stained with Safranin O and Sirius Red. Cartilage proteoglycans in the regenerated tissues from four specimens in 25-mm group were detected by immunoblotting using specific monoclonal antibodies. Type II and type I

collagens and Proteoglycans (PGs), including aggrecan, biglycan and decorin, were detected by immunoblotting with specific antibodies.

**Results:**

Peak vertical ground reaction force (pvGRF) of the operated limb had recovered to an average of 85 % comparing to the contralateral un-operated ones at the end of the study, with no significant difference between groups.

Regenerated tissue was observed overlying the bone on all specimens and was picosirius positive for collagen.

In the load protected group, all 4 operated specimens were Safranin-O negative. In the loaded groups, 4 out of all 6 specimens in each group were Safranin-O positive. In all three loaded groups GAG content in the regenerated tissues was significantly lower than the contralateral control cartilage ( $p < 0.01$ ).

Regenerated tissues from loaded groups showed two clear -chain bands, 1 and 2, at the expected sizes. Western blotting results confirmed a mixture of type II and type I collagens in all regenerated specimens. The presence of cartilage aggrecan, biglycan, and decorin in the loaded groups was also confirmed by immunoblotting.

**Discussion and Conclusion:**

This preliminary study indicates that the cascade of connective tissue differentiation seen in indirect bone repair can be exploited in *in vivo* tissue engineering to resurface synovial joints with *de*

*novo* cartilaginous tissue. Translation of this concept to clinical use could not only provide a biological re-surfacing but may also allow a hemiarthroplasty approach with greater prosthetic longevity in the absence of wear debris and potential issues associated with metal ion release.

Although in early stages of development refinements can be introduced in terms of the compliance of the prosthetic femoral head, also the progression to resurfacing of both articular surfaces.

We conclude that a cartilaginous-like tissue with a mixture of hyaline and fibro cartilage can be regenerated on a bone surface using controlled contact stresses applied from selected prosthetic femoral heads on the acetabular articulation in the ovine hemiarthroplasty model.

The data show the potential for regeneration of hyaline cartilage by *in vivo* tissue engineering using a mechano-biological approach in an integrative physiological environment.

Initial hybridisation of synthetic and autologous biological tissue replacement may allow a more rapid introduction and use of biological constructs in orthopaedic practice.

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