

## **The Role of Mechanobiology in Tissue Engineering**

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Tissue engineering has been defined as 'an interdisciplinary field that applies the principals of engineering and of life science towards the development of biological substitutes that restore, maintain or improve tissue or organ function' by the pioneers in the field, Langer and Vacanti. It is commonly stated that the main components of a tissue engineered construct are:

- 1) cells, to form a biological matrix, which may be differentiated cells from the tissue of interest or stem cells/precursor cells;
- 2) a scaffold on which cells are seeded to maintain shape and structure;
- 3) nutrients which are added to induce the cells to form the appropriate tissue matrix of interest.

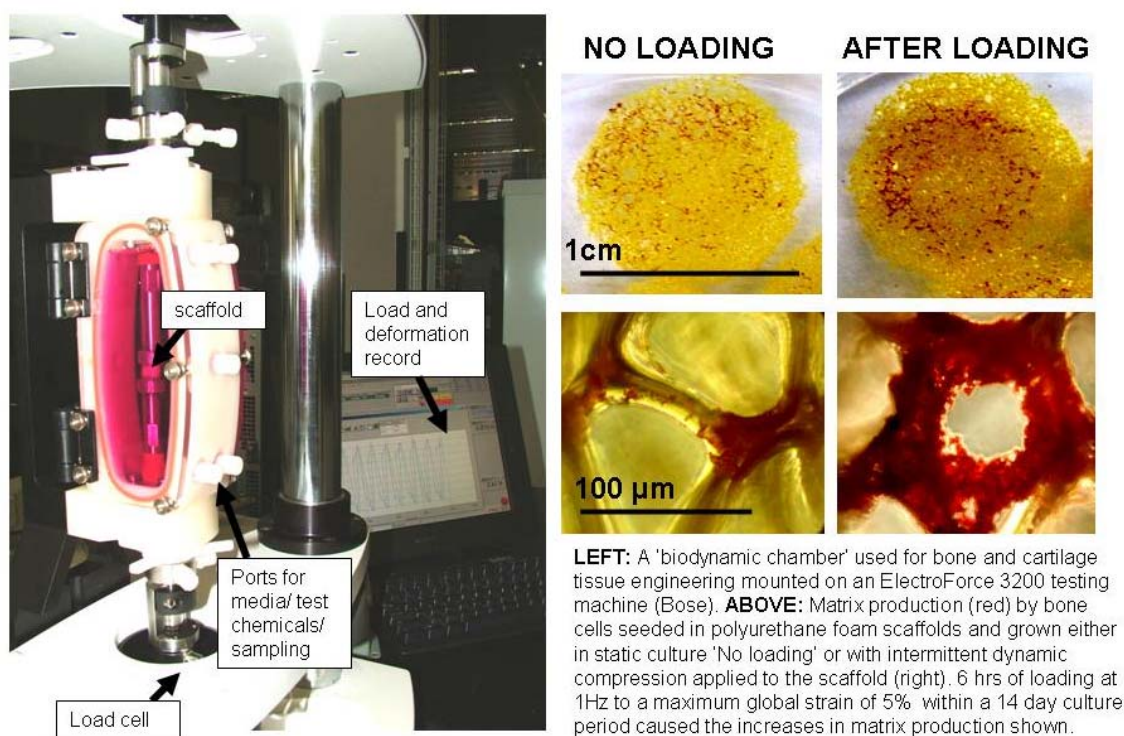
In some tissue engineering concepts the cell-seeded scaffold would be implanted into a patient immediately, but many scenarios involve long-term culture of the cells, either to induce differentiation, or to allow some matrix to be produced prior to implantation for mechanical stability. For long term culture of a cell-scaffold construct it should be grown in an environment conducive to encouraging matrix production - a bioreactor. All cells in the body are subjected to mechanical forces, this is more obvious for tissues involved in locomotion; muscle, tendon bone and cartilage where strains are routinely applied to the tissue. However non-load bearing tissues are also subjected to forces such as fluid flow induced shear stresses and hydrostatic pressure. Therefore it is not surprising to find that cells need a defined mechanical environment in order to produce a functional specified tissue of interest.

In early tissue engineering trials the aim of bioreactor culture was to provide nutrient perfusion to cells in the centre of a thick construct. There were two bioreactors systems, in common usage; rotating wall or perfusion systems. In rotating wall bioreactors samples are suspended in culture medium in a cylindrical chamber and the cylinder rotated at a speed that allows the constructs to fall through the medium but not hit the sides. Perfusion bioreactors involve pumping of nutrient containing medium through the construct. Since these systems apply mechanical forces as well as allowing nutrient movement it is hard to separate the two effects. The stresses and strains applied to the cells are not measurable in these basic bioreactor systems, making it difficult to fine tune the system or understand the mechanism of increased tissue formation.

Mechanobiology is the study of how cells respond to mechanical forces and many cell types have been studied *in vitro* in order to tease out the signalling mechanisms by which a mechanical force results in a biochemical response. For instance, a layer of endothelial (blood vessel lining) cells can be subjected to fluid shear *in vitro* and will respond with changes in cell shape/alignment and production of signalling molecules. Bone cells can be stretched on a membrane and be shown to release factors involved in bone remodelling. Cartilage cells seeded in a gel and subjected to dynamic compression modulate production of the glycosaminoglycans that form the extracellular matrix. The mechanobiology approach is usually to apply precise loading conditions in an environment designed for the assays required (e.g. microscopy, collection of media, matrix analysis). Early mechanobiology experiments were performed on two-dimensional monolayers of cells. However, many studies are emerging which show that there are dramatic differences between how the same cell types behave in a three-dimensional environment compared to monolayer. So mechanobiologists have moved towards applying load in three-dimensions; each investigator usually designs a custom made rig for this purpose and applies load via a stepper motor or a mechanical testing machine.

Recently, the fields of mechanobiology and tissue engineering have come closer together. Tissue engineers are aware that the mechanisms by which cell behaviour is controlled in bioreactor culture need to be better understood in order to create mechanically functional tissues. At the same time mechanobiologists have seen that tissue engineering provides excellent models to study how cells respond to mechanical forces in a relevant three-dimensional environment.

In our laboratory we combine tissue engineering and mechanobiology concepts to study mechanical modulation of bone formation. We use one of the few commercially available sterilisable bioreactor-style chambers specifically designed for use with a mechanical testing machine (Figure 1). The Biodynamic chamber from the ElectroForce systems group of Bose attaches to a Bose bench top testing machine or can be purchased as part of a 'biodynamic test instrument' that fits inside an incubator. Most of our work is conducted in polyurethane foam scaffolds since these have high porosity while maintaining structure, with excellent fatigue resistance and slow degradation. Therefore our scaffold retains mechanical properties and structural integrity over long term culture while allowing cell-synthesised matrix to fill the large pores. We apply dynamic mechanical compression to a cylinder of scaffold thereby applying both strain to the cells' substrate and shear stress via fluid being squeezed in and out of the scaffold. The Biodynamic chamber can also perfuse fluid through the construct to provide continuous flow.



**Figure 1. Biodynamic chamber and cell scaffolds.**

In our system we have shown that short bouts of dynamic mechanical loading increase matrix (collagen and calcium) production by bone cells in a similar way to previous experiments using rotation or perfusion bioreactor culture (Figure 1). The system is very sensitive to changes in the duration of the loading period; repeated 1 hour sessions of loading caused an increase in cell number but not matrix production, whereas 2 hour sessions maintained cell number but increased matrix production. Experiments performed *in vivo* in live animals have shown that only a few cycles of loading can trigger increased bone production. If tissue engineering processes can harness the cells' high sensitivity to the mechanical environment it may be that only short periods of loading are needed during culture. However, it is clear from this sensitivity that the mechanical environment will need to be fine-tuned for each cell and

scaffold type dependant on the ultimate goal of the tissue engineering process (e.g. stimulating cells prior to implantation or production of functional tissue).

The optimum bioreactor for a combined tissue engineering/mechanobiology approach would include a system in which cell responses can be visualised and tissue growth can be monitored in real-time. The cell culture media should be able to be sampled and supplemented without compromising sterility, and for non homogenous scaffolds it is important that local strains and flow can be monitored (or modelled) so that cells' responses can be correlated with the local environment. There is much ongoing work in both bioreactor design and bio-imaging techniques to try to improve on current systems. There is no doubt that the input of mechanical engineers will be critical in the future optimisation of bioreactor design.