

Tissue Engineering of Bone: The role of osteoblasts and osteoclasts

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Bone tissue engineering focuses on the development of osteogenic cell-based therapy to suit multiple clinical requirements for the reconstruction of large orthopaedic defects and to optimise orthopaedic implants. Three factors are essential for bone tissue engineering: 1) the osteogenic stem cells; 2) biocompatible scaffolds which are suitable for osteogenic cell attachment, proliferation and differentiation; 3) a bioenvironments for long-term bone tissue formation and maintenance.

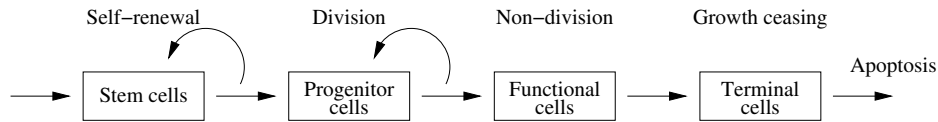
Living bone is a dynamic tissue with modelling and remodelling over life time. Modelling is the process of bone formation by osteoblasts, Osteoblasts produce bone matrix and result in increases of bone mass. Remodelling is the process of bone resorption by osteoclasts. Osteoclasts resorb bone matrix and result in osteocyte (the terminal stage of osteoblasts) apoptosis and decreases of bone mass. In normal condition, the resorption will be followed by new bone formation, to keep the balance of bone mass to suit the change of bone mechanical environment and to repair bone microdamages.

Osteoblasts and osteoclasts play important roles in bone metabolism. The study of osteoblasts and osteoclasts is motivated by almost every bone-related clinical problem, such as bone repair and regeneration (fracture healing), metabolic bone diseases (osteoporosis), bone cancers, and extraskeletal ossification. An understanding of how osteoblasts and osteoclasts maintain a dynamic equilibrium in order to ensure coherent tissue structure is a fundamental question of bone tissue.

Current tissue engineering research is mainly targeting bone forming cells, namely osteogenic stem cell and progenitors which give rise to osteoblasts. These cells have great potential to form mineralised tissue both *in vitro* and *in vivo*. However, as only bone-forming cells are used, it is a static bone modelling system. Once cells are in contact with each other, the cell growth will be ceased and it can not form a self-renewal dynamic system.

At the Botnar Research Centre in Oxford, we are trying to understand the dynamic behaviour between the two cell types using an *in vitro* culture system. However, the major difficulty we are facing is to have a mathematical tool to quantify the two cell populations and to predict cell growth patterns in this dynamic system. This two cell-type co-culture system has been tested in a 2D culture substrate in a pilot experiment; and it is proposed to be tested further in a 3D culture system. In order to understand this two cell-type co-culture system, we first need to understand their behaviour when they are cultured individually.

When osteogenic cells are cultured individually, the population of these cells are dependent on three factors: cell division (proliferation), cell differentiation, and cell death (apoptosis). These processes can be put in the compartment boxes:



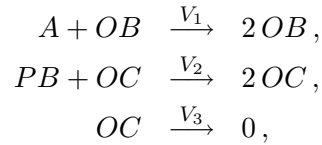
Both osteoblastic and osteoclastic progenitor cells can be grown in larger numbers in a simple two dimensional culture flask nutrient rich assay. It is useful to know the number of cells in a given region over a given period of time. Originally, this would be quite straightforward, however, experimental evidence shows that the distribution of cell proliferation across a 2D surface is heterogeneous. The heterogeneity stems from cell-cell contact inhibition. When a cell is surrounded by three to five other cells, it ceases to proliferate. This heterogeneity commonly leads to open spaces in the cell distribution where no cells have filled the space, that gradually reach total confluence. It is not possible to estimate cell number, either by dividing the total area by average cell area, or by using the unconstrained growth formula:

$$\frac{dX}{dt} = \mu X \quad \Rightarrow \quad X(t) = X_0 \exp(\mu t).$$

The growth rate μ is equal to $\ln 2/t_d$, where t_d is the doubling time. The doubling time of hMSCs under optimal conditions. is between 12 and 24 hours. In reality it is often longer.

The study group is asked to formulate a model that will predict the density of cells in a 2D assay at a given time, taking into account the effect of cell-cell contact. Cell differentiation and apoptosis may need to be considered when predicting the population.

The dynamics of the two-type cell co-culture system will be based on the cell behaviour when they are cultured separately. The co-culture model may be described as:



where OB is osteoblastic cells, OC is osteoclasts, and A is available resources for cell growth. Then V_1 is the velocity constant of OB proliferation, V_2 is the velocity constant for OC proliferation, and V_3 is the velocity constant for natural mortality.

The study group is asked to formulate models to help answer the question of how osteoblasts and osteoclasts maintain a dynamic balance to ensure coherent structure of the formed tissue.

The dynamic behaviour between osteoblasts and osteoclasts is proposed to be studied using a 3D assay. Initially, osteoblasts are grown in a 3D environment. Nutrient is supplied to the cells via hollow fibres, which enter at particular points around the edge of the gel assay.

Once most of the assay region is filled with osteoblasts, osteoclasts are introduced. The osteoclasts burrow into the osteoblast population inducing cell death (apoptosis) as they do so. The osteoblast cells then may migrate and proliferate into the empty region and re-populate the free space, forming new matrix. The osteoclasts continue to proliferate and invade the osteoblast cell population and may also undergo cell death. The dynamics of the two cell types is such that overall bone tissue structure is not compromised during the process. In order to ensure that the formation of homogeneous bone tissue, it is important that waste products from the cells as well as debris from the apoptotic process are removed.

The study group was asked to formulate models to help answer the following questions:

1. How do osteoblasts and osteoclasts maintain a dynamic cell balance to ensure coherent structure of the formed tissue?
2. What role does cell migration versus cell proliferation play in maintaining this balance?
3. What is the optimum method for removing cell waste products from the tissue structure, e.g. diffusion?