EVALUATING OSTEOINDUCTIVE AND OSTEOCONDUCTIVE PROPERTIES OF BIOMATERIALS: SELECTION AND USE OF ASSAYS, CELLS, AND CELL SEEDING METHODS

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There are many different biomaterials for bone defect reconstruction, and in vitro screening of these biomaterials for their osteoinductive and osteoconductive properties before implantation in the body requires precise evaluation methods. iFyber provides expertise in the selection of assays, cells, and cell seeding methods for this purpose, as well as expertise in the performance of these assays and techniques.

TYPES OF BIOMATERIALS USED IN BONE RECONSTRUCTION

Bone defects that occur from surgery, trauma, or cancer are often reconstructed using autografts, allografts, xenografts, alloplasts, or implants. As shown in Figure 1, different biomaterials can have varying osteoinductive and osteoconductive properties.

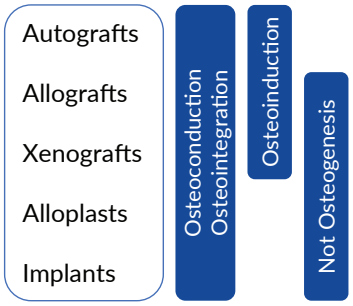
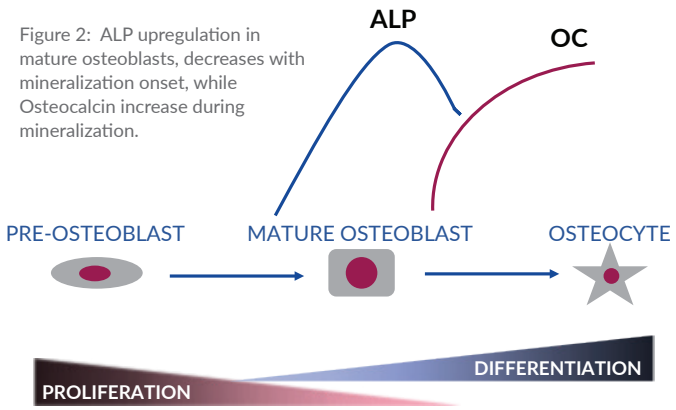


Figure 1: Allograft and xenograft bone are decellularized, may be demineralized/deproteinized, and have osteoconductive and possibly osteoinductive properties. Alloplasts and implants are typically only osteoconductive, except when treated with particular products, peptides, or growth factors

THE BONE REGENERATION PROCESS

Following implantation, a biomaterial comes into contact with preosteoblast cells of its host that exhibit a temporal pattern of gene expression reflecting three significant periods of cell development: proliferation, matrix maturation, and mineralization – where the latter occurs when the osteoblast matures into an osteocyte within the newly mineralizing matrix (Figure 2). Some biomaterials are both osteoinductive and osteoconductive, which means that they contain growth factors that cause the migration of progenitor cells and their differentiation into osteoblasts while providing a suitable substrate for bone formation. On the other hand, osteoconductive biomaterials are not capable of inducing cell differentiation and can only be a substrate for bone formation.

Figure 2: ALP upregulation in mature osteoblasts, decreases with mineralization onset, while Osteocalcin increase during mineralization.



IFYBER'S ASSAY EXPERTISE

iFyber utilizes a number of assays and techniques to assess a biomaterial's osteoinductive and osteoconductive properties. iFyber has expertise in the following assays: determining cell recruitment, attachment, proliferation, and differentiation. The particular assay and techniques used should be tailored to the biomaterial being evaluated and the scientific question. iFyber also has the expertise to assist in making the appropriate assay selections.

An osteoinductive biomaterial should be capable of recruiting osteoprogenitor cells while not causing the migration of inflammatory cells. Cell recruitment patterns and migration depend on many growth factors and cytokines released from the biomaterial. Relevant growth factors and biomarkers of a biomaterial can be evaluated using a variety of commercially available assays. These assays can help guide the development of biomaterials with favorable osteoinductivity and modulated immune responses. The morphology of cells attached to biomaterial surfaces can also be evaluated by various microscopy techniques, including scanning electron microscopy (SEM), bright-field, or fluorescence optical microscopy with the aid of chemical stains (e.g., live/dead staining). Further, cell proliferation can be assessed by measuring total DNA or cell metabolism, while cell migration/recruitment can be assessed by scratch assay (Figure 3).

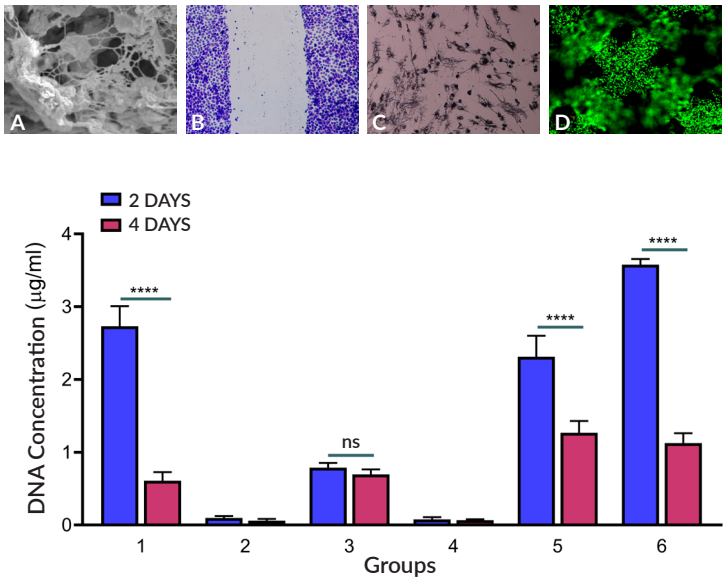


Figure 3: Various assays for evaluating cell attachment/viability/proliferation: a) cells on the surface of a material visualized by SEM, b) Scratch assay (cell recruitment), c) MTT assay, d) Live/Dead staining, e) Total DNA measurement,

The proliferation process ends when osteoprogenitor cells start to differentiate into osteoblasts, and the expression of the genes required for the maturation of bone extracellular matrix, for example, alkaline phosphatase (ALP), is up-regulated. ALP is an early marker of osteoblast differentiation; its activity increases as osteoblasts mature and decreases as mineralization starts. ALP activity assay allows for rapid identification of osteoinductive properties of biomaterials, as shown in Figure 4. The assay can be adapted to evaluate the different concentrations of a product or the effects of several biomaterials (Figure 4).

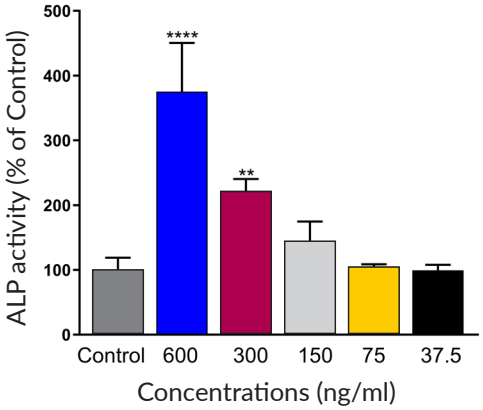


Figure 4: Effect of different concentrations of a product on ALP activity

While ALP is an early marker of osteogenic differentiation, other osteogenic-specific markers, such as OC, which is the late marker of osteoblastic differentiation, should be measured to confirm the presence of mature osteoblasts. Alizarin red S staining can also be performed to visualize the mineralization nodules and detect calcium-containing osteocytes in later stages of differentiation. On the other hand, histology staining (H&E) and SEM can detect the star-shaped morphology of osteocytes and their presence in lacunas (Figure 5).

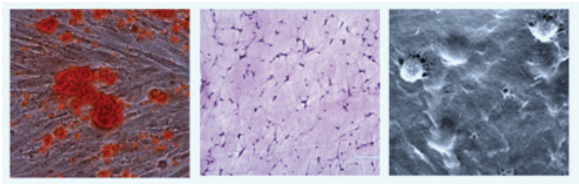


Figure 5: Visualisation of calcium nodules, cell morphology, and osteocytes' lacuna in the last step of differentiation bu using Alizarin Red staining, H&E staining, SEM

CELL SELECTION

iFyber utilizes various types of cells in the abovementioned assays: Primary human osteoblast cells (Hob); human cell lines (SaOs2: mature osteoblasts, MG-63: immature osteoblasts, HfOb: clonal human osteoprogenitor cells derived from natural tissue); mouse cell lines (MC3T3-E1: preosteoblast derived from natural tissue, C2C12: murine premyoblast cell line); and more recently, stem cells (Figure 6).

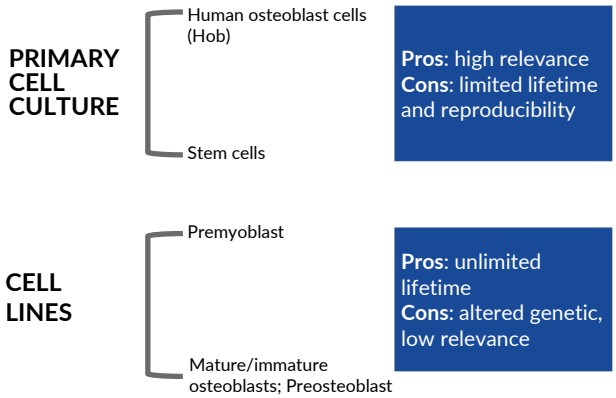


Figure 6: Various types of cells that can be used for evaluating the osteoinductive/ osteoconductive properties of biomaterials

The type of biomaterial being studied is one of the primary determining factors in cell selection for a given assay. While osteoinductive biomaterials should induce stem cell migration, differentiation to osteoblast, and bone formation, an osteoconductive graft requires the presence of existing bone or differentiated mesenchymal cells to encourage bone growth across its surface.

Another important consideration for cell selection is the specific research question. Cells selected for evaluating attachment and proliferation might differ from those used for assessing differentiation and mineralization.

Finally, the timing of the biological process may impact decision-making around cell selection. A specific medium can trigger the differentiation of stem cells in 14-28 days and cell lines in 7 days, whereas a growth factor may cause a fast osteoblastic differentiation (48h) of C2C12 cells.

In the end, primary cell cultures are more relevant than cell lines; however, both could be used to assess biomaterials based on the specific research question.

CELL SEEDING METHODS

Two other important aspects to consider in an assay for evaluating a biomaterial are the preconditioning of the biomaterial and the method of cell seeding, which may affect the assay time, cell proliferation, and differentiation (e.g., the signal

level of biomarkers). iFyber offers two methods of cell seeding. A standard method of cell culturing is 2D culture on an assay plate and either direct contact with the biomaterial (maybe preconditioned) or indirect contact through a filter/barrier or the extract of the biomaterial. Cells can also be seeded on the surface of biomaterials for 3D cell culture. Figure 7 shows different seeding scenarios for evaluating the interaction between cells and biomaterials; the selection of the method depends on the assay's purpose.

In summary, for a detailed and thorough analysis of your biomaterials, iFyber can assist in developing and executing a relevant study design, selecting proper assays, cells, cell seeding methods, and control groups based on the structure and properties of your samples

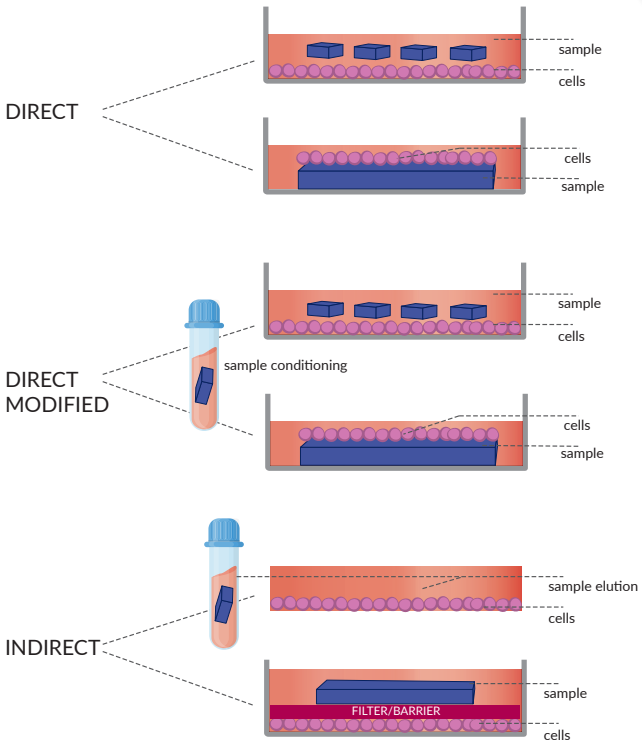


Figure 7: Cell seeding methods and cell/biomaterial (sample) interaction.

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