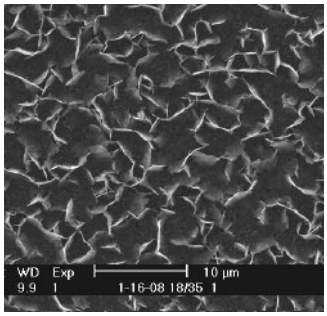
Corning® Osteo Assay Surface 24 Well Plates with Transwell® Permeable Supports – A Useful Tool for Co-Culture Studies



A brief technical report demonstrating Corning Osteo Assay Surface and Transwell Permeable Support compatibility

Rebecca M. Wood and Mark Rothenberg
Corning Incorporated
Kennebunk, ME

Introduction

Bone is composed of organic and inorganic molecules that are in a constant state of flux in order to preserve the integrity of the skeletal system. This system is composed of two major cell types which regulate this dynamic environment: osteoblasts, which synthesize, and osteoclasts, which resorb bone-matrix. The regulation involved in balancing these activities is critical. Any imbalance in the system can result in a number of different problems, the least of which includes osteoporosis (1). Historically, studying bone has been difficult due to limitations in available technologies and methods. Researchers continue to use bone chips from whales or cows as a substrate to culture and differentiate their bone-derived cell lines due to the physiologic relevance of the substrate. Bone chips can be difficult to obtain, very inconsistent and difficult to work with due to handling and issues of visualization related to cells, as well as pit formation on the surface.

In an effort to develop tools designed to enable scientific investigation of bone cell physiology, a study was peformed to evaluate functional osteoclast activity *in vitro* using a new proprietary synthetic inorganic bone surface. The consistency and superior visualization properties of the Corning Osteo Assay Surface were easily demonstrated with the evaluation of osteoclast differentiation and functional pit formation. Studies demonstrating cell-cell communication in an *in vitro* co-culture environment were also performed using this surface in conjunction with the Transwell permeable support insert.

Using the murine macrophage-derived osteoclast line RAW267.4, the effect on osteoclast differentiation and functional activity of cells, incubated with either the RANK Ligand (RANKL) producing cell line MCF-7 or the 5/9 m alpha3-18 cell line, was examined. The 5/9 m line is a constitutive expresser of human macrophage colony stimulating factor (M-CSF). Also, the impact of the human osteoblast osteocarcinoma-Saos-2 line, which is able to secrete RANKL, on RAW267.4 differentiation, was examined (2).

Materials/Methods

Osteoclast Functionality

Tartrate-resistant acid phosphatase (TRAP) activity of RAW 264.7 cells (ATCC Cat. No.TIB-71) grown on the 24 well Corning Osteo Assay Surface plates (Cat. No. 3987) was examined during co-culture with three different cell lines on 0.4 µm pore membrane polycarbonate Transwell Permeable Support inserts (Corning Cat. No. 3413). TRAP activity is a metric used to quantify osteoclast differentiation and function. MCF-7 (ATCC Cat. No. HTB-22™), Saos-2 (ATCC Cat. No. HTB-85™), 5/9 m alpha 3-18 (ATCC Cat. No. CRL-10154™), and RAW 264.7 cells were routinely cultured in IMDM (Mediatech Inc. Cat. No.10-016-CV) containing 10% fetal bovine serum (FBS) (PAA Laboratories Cat. No. A15-201), and harvested with HyQTase™ (Fisher Cat. No. SV30030.01). On the day of seeding, MCF-7, Saos-2, and 5/9 alpha cells were harvested and resuspended in 10% FBS containing MEM (Mediatech Cat. No.10-010-CM). Cells were seeded at 1,000 cells/Transwell insert in a 24 well tissue culture treated plate (Corning Cat. No. 3527). Cells were allowed to grow for seven days with media changed on days 4 and 7. On day 7, RAW 264.7 cells, passage 4 or earlier, were harvested and resuspended in 10% FBS alpha MEM (Mediatech Cat. No. 10-022-CV) containing 50 ng/mL RANKL (Shenandoah Biotechnology Cat. No. 200-04), and seeded at 5,000 cells/well in a Corning Osteo Assay Surface plate. Transwell inserts containing the cell lines previously grown for 7 days were transferred to the 24 well Corning Osteo Assay Surface plates containing RAW 264.7 cells. Cells in upper and lower chambers were allowed to grow for an

CORNING

additional 7 days with a media change on day 4 (10% alpha MEM containing 50 ng/mL RANKL). The culture period chosen was based on previously published research in order to achieve optimal cytokine production (3). On day 7, inserts were removed and discarded, and medium from the Corning® Osteo Assay Surface plate was sampled for TRAP activity following the manufacturers protocol (B-Bridge Cat. No. AK04). As an internal control, RAW267.4 cells were also differentiated without additional cell lines but in the presence of RANKL. Differentiated osteoclasts were stained using the Millipore® Actin Cytoskeleton and Focal Adhesion Staining Kit (Millipore Cat. No. FAK100) for both actin and nuclear localization. Cells were visualized using the EVOS® *fl* microscope (AMG Advanced Microscopy Group).

Results and Discussion

To establish the feasibility of using a co-culturing technique to study bone cell physiology, two unique platforms from Corning Life Sciences were utilized. The study used the Corning Osteo Assay Surface in conjunction with Transwell® permeable supports. The impact of three cell lines (MCF-7, 5/9 m alpha 3-18 and the Saos-1) on osteoclast differentiation was examined. The three lines are known to express important regulators of osteoclast physiology. Figure 1 is a diagram of how the co-culture system was set up. Figure 2 depicts the result of a 7-day incubation of RAW267.4 cultured on the Corning Osteo Assay Surface with MCCF-7, 5/9 m alpha 3-18 and Saos-2 cell lines cultured on the Apical 0.4 µm pore size membrane. At the end of the osteoclast differentiation period, the inserts containing cells were removed and TRAP activity of the multiple well plates was analyzed using the protocol provided by B-bridge. The results (Table 1) show that the 5/9 m alpha 3-18 cell line, a commercially available CHO line that constitutively expresses M-CSF, inhibits TRAP activity (Figure 2, 49 ±1%). The data, expressed as Percent TRAP activity with differentiated RAW267.4 as the control, showed no change in RAW267.4 activity when co-cultured with the MCF-7 cell line (100 ±1%). The human osteosarcoma line, Saos-2, which under the appropriate conditions can differentiate into osteoblasts and express RANKL (2), showed enhanced RAW264.7 differentiation and TRAP activity (147 ±5%). These data indicate that osteoclast physiology can be studied and modified using this co-culture system. Figure 3A and B show differentiated RAW267.4

Table 1. Percent TRAP Activity of Differentiated RAW264.7 Cells After Co-culture with Three Different Cell Lines

	Overall (%)	Standard Deviation
MCF-7 + RankL	102	0.1
5/9 alpha + RankL	45	0.1
Saos-2 + RankL	141	0.5

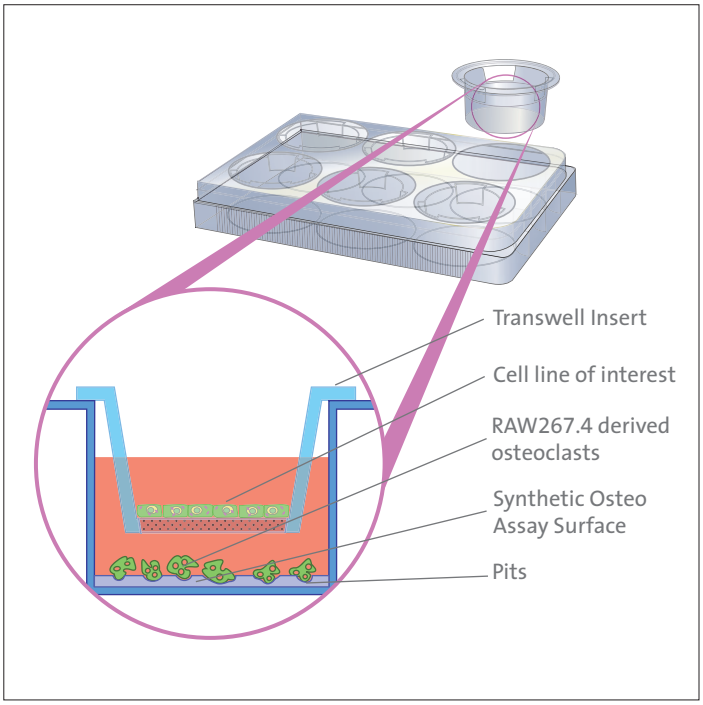


Figure 1. Schematic of a Transwell Permeable Support – Corning Osteo Assay Surface Co-Culture System

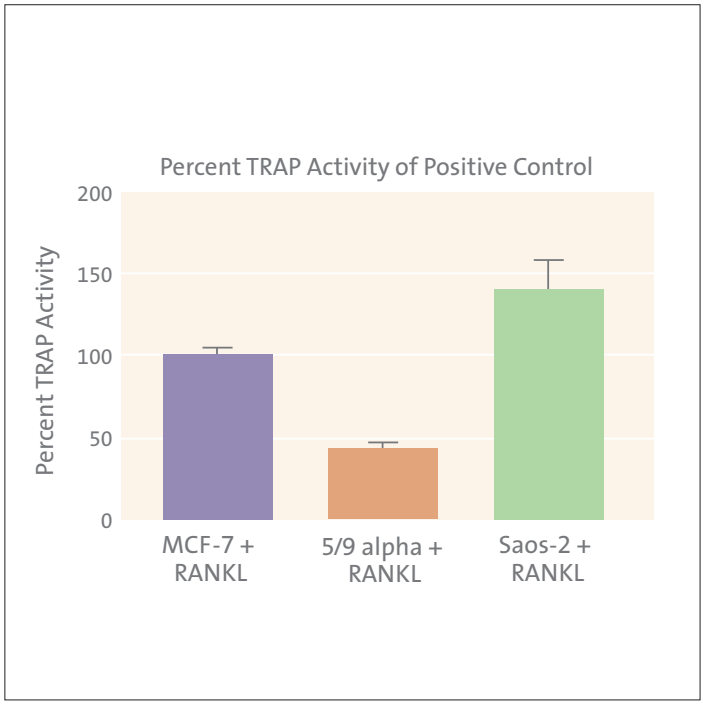


Figure 2. Percent TRAP activity of co-cultures expressed as compared to control wells containing RAW 264.7 cells in the presence of RANKL. (n=3)

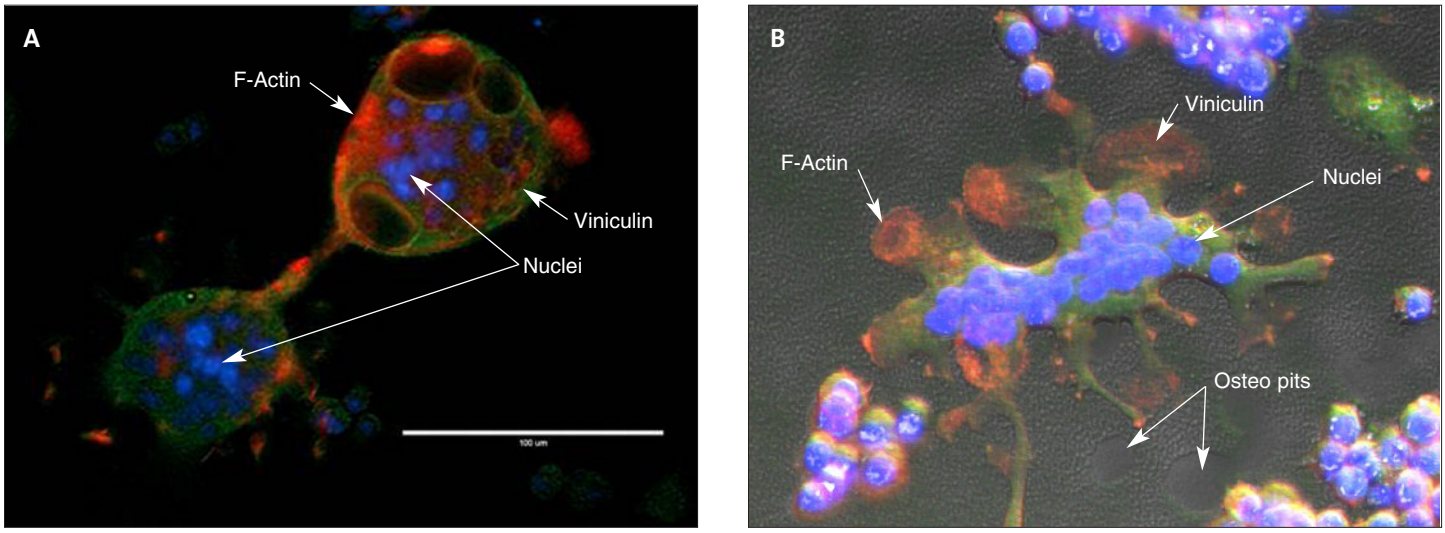


Figure 3. RAW264.7 cells were grown for 7 days in the presence of RANKL on Corning Osteo Assay Surface 24 well plates. Cells were fixed and stained using an Actin/Cytoskeleton and Focal Adhesion Staining Kit (Millipore) and visualized using an EVOS® *fl* microscope. Figures 3A and B are representative osetoclasts stained using the same protocol.

cells stained with Rhodamine-Phalloidin, DAPI, and Vinculin antibodies to reveal the osteoclast-like structure of the cells after 7 days of differentiation. The observed staining and characteristic multinucleated structures present are indicative of an osteoclast cell. Pit formation can also be observed in Figure 3B which correlated with the TRAP data (data not shown).

This type of co-culture experimental design can also be used to examine cellular migration. Figure 4 is an example of how such an experiment can be set up to examine how osteoclasts or osteoblasts modulate cancer cell (e.g., breast or prostate) migration *ex vivo*.

Conclusions

- Utilizing Corning® 0.4 µm pore size membrane Transwell® Permeable Supports and the new Corning Osteo Assay Surface technology, co-culture studies can be conducted to better understand the effects on osteoclast differentiation and activity.
- The Corning Osteo Assay Surface can be used with fluorescent techniques to examine cellular markers of differentiation.
- The Transwell Permeable Support-Corning Osteo Assay Surface system can be used for examination of a number of interesting physiologic events, including, but not limited to, migration of various cell lines where bone or bone-related cells may play a role.

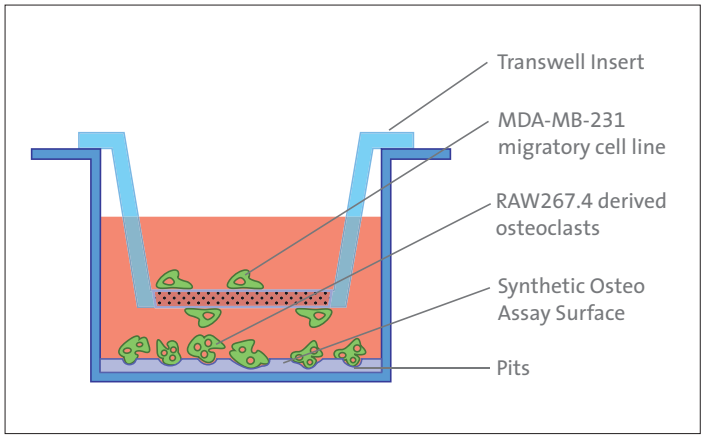


Figure 4. Schematic showing the use of the Transwell Permeable Support system with the Corning Osteo Assay Surface for migration studies.

References

1. Zhao, W. et al. 2009. The Role of T Cells in Osteoporosis, an Update. Int. J. Clin. Exp. Pathol. 2:544-552.
2. Hofbauer, L.C. et al. 2008. Fatal attraction: why breast cancer cells home to bone. Breast Cancer Research 10:101.
3. Nicolin, V. et al. 2008. Breast adenocarcinoma MCF-7 cell line induces spontaneous osteoclastogenesis via a RANK-ligand-dependent pathway. Acta Histochemica Vol. 110(5):388-396.

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, please call +1.978.442.2200.

CORNING

Corning Incorporated
Life Sciences
Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 0402-794-347

China
t 86 21 2215 2888
f 86 21 6215 2988

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

Corning Incorporated, One Riverfront Plaza, Corning, NY 14831–0001