# Investigating Cellular Patterning of Breast Cancer cells with ADGRF1 Overexpression or Activation on Novel Micropatterned Substrates

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## Introduction

- G protein-coupled receptors (GPCRs) are known to be excellent drug targets; however, the second largest family of adhesion-GPCRs are yet unexplored for their role in health and disease.
- ADGRF1 (previously known as GPR110) is an adhesion-GPCR that has an important function in neurodevelopment, and its high expression is a known predictor of poor survival in cancer. However, the downstream pathways of ADGRF1 remain largely unknown in cancer.
- Recently the effects of ADGRF1 overexpression (OE) on tumorigenesis and signaling pathways have been reported in human epidermal growth factor receptor-2-positive (HER2+) breast cancer (BC).
- The interrogation of clinical data also showed that ADGRF1 is overexpressed in HER2+ BC subtype and predicts worse BC-specific and overall survival in these patients.
- In vitro models of cellular patterning in human tumor development are invaluable in the study of BC.
- The use of a novel silicon (Si) titanium diboride ($TiB_2$) micropatterned substrate platform may help further characterize the cellular effects of ADGRF1 OE or activation.

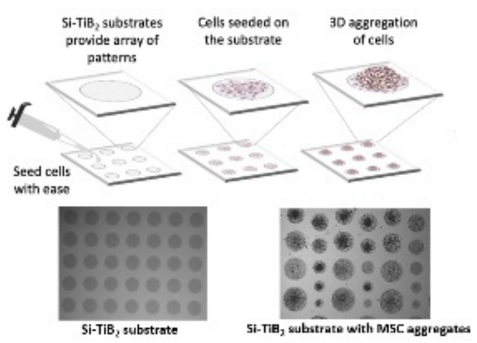


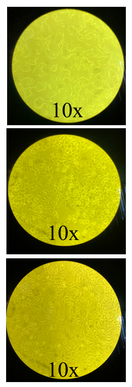

Figure 1. Novel Si_$TiB_2$ micropatterned substrate platform

## Aims

In this study, we aimed to use novel silicon titanium diboride (Si_$TiB_2$) micropatterned substrates to study the cellular patterning associated with ADGRF1 overexpression in HER2+ BC as well as ADGRF1 activation by agonist (Synaptamide) in triple-negative BC.

## Materials

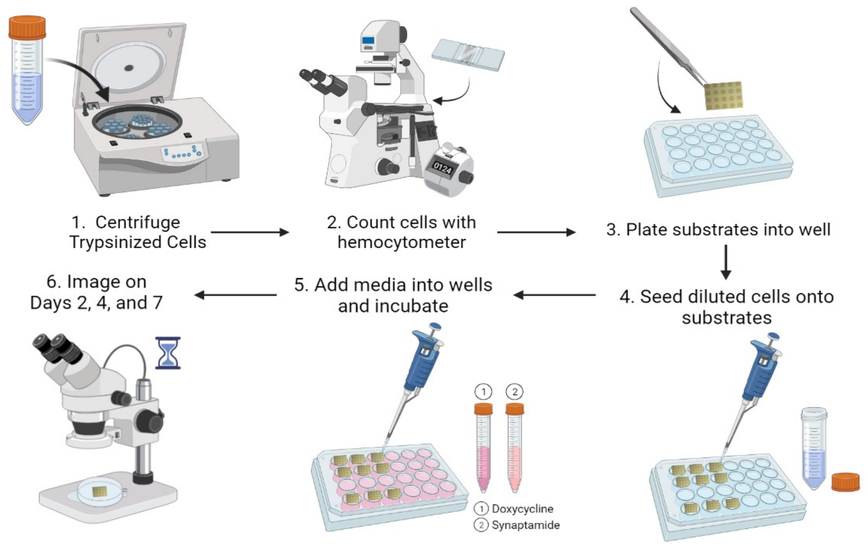
| | | | |
|---|---|---|---|
| CELL LINES | BT474 Pool | HER2+/HR+ | Stable pool cells contain Tet-On Dox-inducible pHAGE lentiviral system with ADGRF1 cDNA |
| | SKBR3 Pool | HER2+/HR- | |
| | MDA-MB-231 | HER2-/HR- | Triple negative BC with endogenous ADGRF1 OE |
| DRUGS | Doxycycline | - Induce ADGRF1 OE of BT474 & SKBR3 Pool<br>- Concentrations used: 0.2 and 2ug/ml | |
| | Synaptamide | - ADGRF1 agonist<br>- Concentrations used: 1 and 10nM | |
| SEEDING CELL DENSITY: 300 cells/mm$^2$ for all cell lines | | | |




## Methods

- Platform development: Circular $TiB_2$ layers are deposited on Si substrates using electron-beam evaporation. Photolithography is then used to micropattern the substrates.
- Cellular patterning on the micropatterned substrate is mediated via differences in stiffness, hardness, hydrophilicity, and surface charge across Si and $TiB_2$ coupled with selective adsorption of growth factors with heparin-binding domains (e.g., Fibroblast Growth Factor (FGF)). FGF is selectively adsorbed on $TiB_2$ patterns in the presence of heparin which facilitates cell adhesion on the patterns.

Seeding Breast Cancer Cells onto Novel Si_$TiB_2$ Substrates:

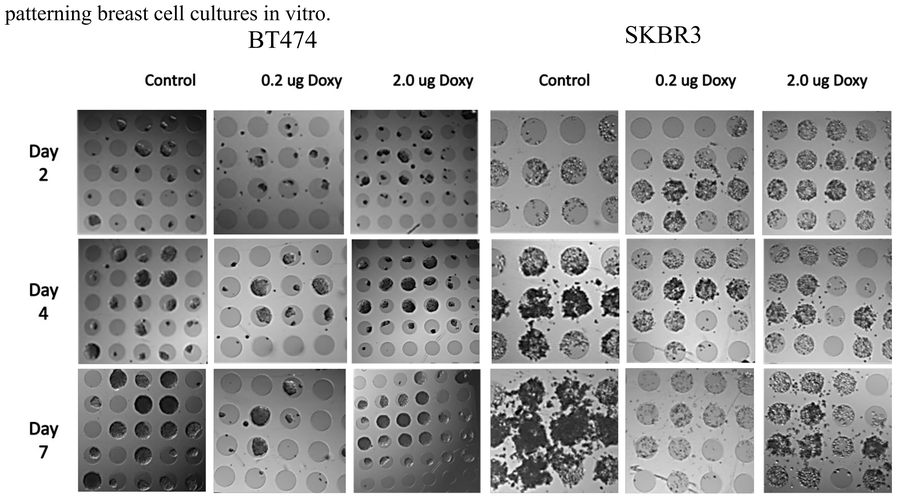



Doxycycline was added to media on Day 0 for BT474 and SKBR3 Pool. Synaptamide was added to media on Day 2 for MDA-MB-231. Cell Density for all cell lines: 300 cells/mm$^2$.

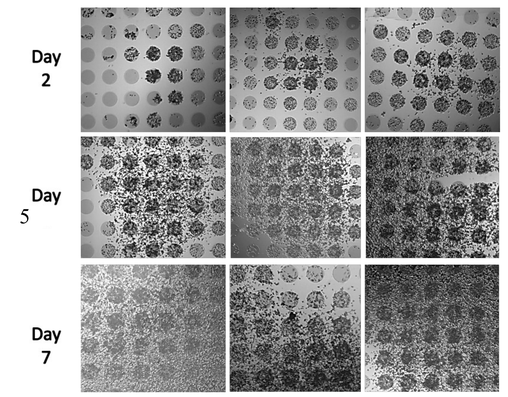
## Results

In our research, the following observations were made:

1. There was visible growth at a dilution of 300cell/mm$^2$ for all cell lines regardless of ADGRF1 receptor activation by Doxycycline and Synaptamide.
2. Both MDA-MB-231 and SKBR3 Pool showed gradual migration from $TiB_2$ to the background Si while BT474 cells showed selective aggregation limited to $TiB_2$.
3. Despite observing cell growth in MDA-MB-231 conditions with Synaptamide, the agonist's effects requires additional investigation.
4. Our study supports the potential of the microfabricated Si_$TiB_2$ substrate platform to be used for patterning breast cell cultures in vitro.




MDA-MB-231




## Discussion

This preliminary data is the stepping-stone for future studies which include viability assays, qPCR to explore receptor biomarker activity, replicating more trials to obtain statistical analysis, and testing seeded substrates with anti-cancer drugs such as Lapatinib for BT474 and SKBR3 and Docetaxel for MDA-MB-231. In addition to BC cell lines, further testing with other cancer types will be conducted to determine the consistency of cellular growth patterning on the Si_$TiB_2$ substrates. Through continued research, these novel substrates could pave a pathway for a cheaper in vitro alternative to studying cancer cells.

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