

Culture of the IDG-SW3 osteocyte cell line.



Bethan K Davies, MBio
Department of Comparative Biomedical Sciences, Royal Veterinary College, London, NW1 0TU, UK.
Email: bkdavies@rvc.ac.uk

Isabel R Orriss, BSc, Ph.D.
Department of Comparative Biomedical Sciences, Royal Veterinary College, London, NW1 0TU, UK.
Email: iorriss@rvc.ac.uk



The IDG-SW3 osteoblast-to-late-osteocyte cell line is derived from a temperature-sensitive DMP1-GFP transgenic mouse. IDG-SW3s cultured with interferon γ (IFN γ) at 33°C will proliferate, whilst culture in mineralising conditions without IFN γ at 37°C enhances differentiation. This HubLE Method describes the protocol for culturing this cell line [1].

Materials

- Rat-tail type 1 collagen
- Recombinant mouse interferon γ (IFN γ)
- Phosphate buffered saline (PBS)
- Proliferation medium Alpha Modified Essential Medium (Prolif α MEM) [**Tip No. 1**]
- Osteocyte-differentiation α MEM (OcyMEM) [**Tip No. 2**]
- Trypsin-EDTA (0.25%)
- Glutaraldehyde (2.5%)
- Alizarin Red (1%)

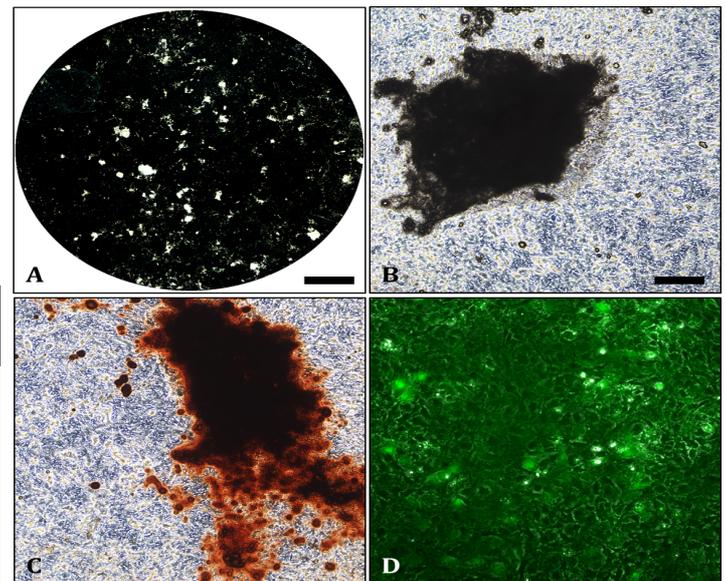


Fig. 1. (A) Whole well scan and phase contrast microscopy images of (B) unstained and (C) alizarin red stained IDG-SW3 cells at day 30 of culture. (D) DMP1-GFP expression at day 14. Scale bars: whole well = 0.5cm, phase contrast images = 200 μ m.

Method

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All procedures should be performed under sterile conditions.

1. Coat all required tissue culture plastics for 1 hour at room temperature with 0.15mg/ml of rat-tail type 1 collagen in 0.02M acetic acid.
2. Remove the collagen solution and either wash with PBS for immediate use, or air dry plates prior to storage [**Tip No. 3**].
3. Thaw a vial of IDG-SW3 cells into 5ml Prolif α MEM and spin at 1,500 rpm for 5 minutes.
4. Remove the supernatant, re-suspend the pellet in Prolif α MEM and seed into a collagen-coated 75cm² flask containing Prolif α MEM. Incubate at 33°C with 5% CO₂.
5. Once \geq 80% confluent (2-3 days post-seeding), remove medium, wash with PBS and incubate with 0.25% Trypsin for 5-10 minutes to detach cells.
6. Spin at 1,500g for 5 minutes and re-suspend in Prolif α MEM (1ml per flask).
7. Seed cells in collagen-coated tissue culture trays or flasks (for further expansion and use of cells at next passage) in Prolif α MEM [**Tip No. 4**].
8. Incubate at 33°C with 5% CO₂ until confluent.
9. At this stage, remove the Prolif α MEM medium, carefully wash the cell monolayers with PBS and add OcyMEM. Incubate at 37°C with 5% CO₂.
10. Culture for up to 30-35 days with half medium changes of OcyMEM every 2-3 days. Mineralisation is usually evident from \sim 10-14 days.
11. Fix with 2.5% glutaraldehyde for 5 minutes before staining with 1% alizarin red to visualise mineralised nodules [**Tip No. 5**].

Tips

[Update](#)

1. **ProlifαMEM:** Add 10% heat-inactivated foetal calf serum (FCS), AB/AM (100U/ml penicillin, 100µg/ml streptomycin, 0.25µg/ml amphotericin) and L-glutamine (200mM). Aliquot the stock media and add IFN γ (2500U/ µl). Incubate the medium at 33°C prior to use and limit exposure to heat due to IFN γ degradation.
2. **OcyMEM:** Add 10% heat-inactivated foetal calf serum (FCS), AB/AM (100U/ml penicillin, 100µg/ml streptomycin, 0.25µg/ml amphotericin) and L-glutamine (200mM). Add 50µg/ml ascorbate and 2-4mM β -glycerophosphate (the original paper uses 4mM β -GP ^[1], however, IDG-SW3 cells differentiate and mineralise sufficiently in 2mM). Always make fresh on the day of use.
3. **Collagen-coated tissue culture plates/ flasks:** Ensure all plates are coated under sterile conditions in a tissue culture hood. Use a cold pipette (stored in the freezer until use) to stop the collagen sticking to the plastic. The 0.15mg/ml collagen solution can be re-used 5-6 times; coating for ~1 hour each time. Coated plastics wrapped in parafilm can be stored at 4°C for up to 6 months until use.
4. **Seeding density:** IDG-SW3 cells will mineralise in 12 and 6-well plates but due to the long culture duration some monolayer peeling should be expected. Woo *et al.* ^[1] recommend seeding IDG-SW3 cells at 4x10⁴ cells/cm², although the lower densities of 10⁴ (12-well) and 10⁵ (6-well) will also support osteocyte proliferation, mineralisation and differentiation. To expand IDG-SW3 cells for the subsequent passage seed at 5x10⁵ cells/ 75cm² flask.
5. **Alizarin Red staining:** Mineralised bone nodules can be stained with alizarin red (Fig.1C). It is also possible to obtain good quality images on unstained cell layers (Fig 1A-1B). DMP1-GFP expression can be visually monitored throughout the differentiation process (Fig.1D). Evaluation of E11, DMP1 and sclerostin gene/protein expression is also advisable to confirm osteocyte differentiation.

References

[Update](#)

1. Woo SM, Rosser J, Dusevich V, Kalajzic I, Bonewald LF (2011). Cell line IDG-SW3 replicates osteoblast-to-late osteocyte differentiation *in vitro* and accelerates bone formation *in vivo*. *J Bone Mineral Res* 26:2634-2646.