Testing Cell Transplantation Methods in the Planarian *Schmidtea mediterranea*

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Following amputation *Schmidtea mediterranea* can regenerate any missing tissue necessary to restore full function to the animal. Regeneration proceeds at the wound site when an aggregation of neoblasts, known as a blastema, is formed. Neoblasts are planarian stem cells and are the only proliferating population in the animal. They are believed to be pluripotent and necessary for planarian regeneration.

These cells are constantly entering the cell cycle and therefore can be eliminated through irradiation. Irradiated animals were amputated producing tail fragments and prepharyngeal (no head) fragments. These fragments were injected with neoblast fractions acquired through fluorescence activated cell sorting (FACS) in two radio-sensitive groups. Following injection, fragments were fixed daily over a 6-day time course. Fixed fragments were stained with H3P antibody and *Tyramide* signal amplification to observe the mitotic activity of the injected neoblasts. *Tyramide* signal was visible in the unirradiated positive control animals only; no fragments had visible signal.

Two possible explanations are:

1) current injection protocol is insufficient and neoblasts are not entering the fragments.

2) Neoblasts are dying before or shortly after injection; possibly due to exposure during purification and/or injection.

Further experimentation should be done. Fluorescent marking of injected cells is needed to determine where the cells are entering the animal fragments and whether or not they are remaining there.

Optimization of future studies will require testing injections of cells at various times after irradiation and amputation. This would allow us to test if the proliferation signals from the wound site are cumulative. If so, increased levels of these factors may improve our chances of triggering mitoses in the injected cell population.