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for high resolution imaging of specifically labelled cell populations. As an alternative to using unpurified OPN^+^ OS cells, we were able to enrich for the OPN-expressing cells, by first generating a density gradient using Percoll. Next, we used a modified fluorescence activated cell sorting protocol which included a suspension step in order to enrich for primary human OS cells and to improve OPN immunofluorescence signal

(magnification x1000). After the enrichment process, a range of primary cell numbers were verified with fluorescence microscopy and flow cytometry. Normal human bone marrow cells were used as a positive control for OPN immunostaining. As a negative control, no OPN staining was detected in the negative control sample, when used at the same fluorescent settings to the OPN^+ sample ([Figure 7](

#pone-0076234-g007){reftype="fig"}). These results showed that by using a density gradient and cell enrichment techniques, it was possible to specifically identify and isolate OPN^+^ OS cells from their surrounding bone marrow fraction. ![OPN is expressed in primary OS cells.\ Normal human bone marrow (BM) cells were used as a positive control for OPN immunostaining. No staining was detected in the negative

control sample, when used at the same fluorescent settings to the OPN^+ sample.](pon e.0076234.g007){#pone-007 6234-g007} It is thought that OS cells have a long half-life *in vivo* and that OPN may be involved in the recruitment of other cells types, including inflammatory cells, to the site of a developing bone lesion [@pone.0076234-Sternheim1]. As OS cells are likely to have longer half-lives *in vivo*, the enrichment technique presented here could be used

with the aim of differentiating primary OS cells, from c6a93da74d

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