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Foreign Body Giant Cells (FBGC) overexpress OC-STAMP and its inhibition impedes cell fusion

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Background: Osteoclasts are multinucleated bone resorbing cells that have an important role in bone remodeling. Osteoclast precursors are mononuclear and are derived from bone marrow, they circulate in the blood and are induced, in the presence of RANKL, into multinucleated cell that have a capacity to resorb bone (1). Fusion of monocyte/macrophage lineage cells, a complicated process and yet to be fully understood, leads to activation of various cell surface receptors like SIRP α (2), CD44 (3), DC-STAMP (4) and recently discovered OC-STAMP (5). OC-STAMP is an important Trans-membrane protein that is highly up-regulated during osteoclast formation, OC-STAMP over expression increases osteoclast formation but its inhibition also abrogates osteoclast formation and function. Bone marrow derived monocyte/macrophage cells also have the capacity to fuse under the influence of IL4 into FBGCs (6). IL4 also inhibits osteoclast formation and hence leads to differentiation into other cell lineages (7). FBGCs are usually found at the tissue and material interface like prosthesis, implants and medical devices. They play an active role in the foreign body reaction and not only hinder the osteointegration of various implants (8), but also total rejection of the medical devices (9). As these giant cells are implicated in many clinical conditions, understanding the mechanism of formation and subsequent function can provide valuable information which can be used control to FBGCs.

Results:

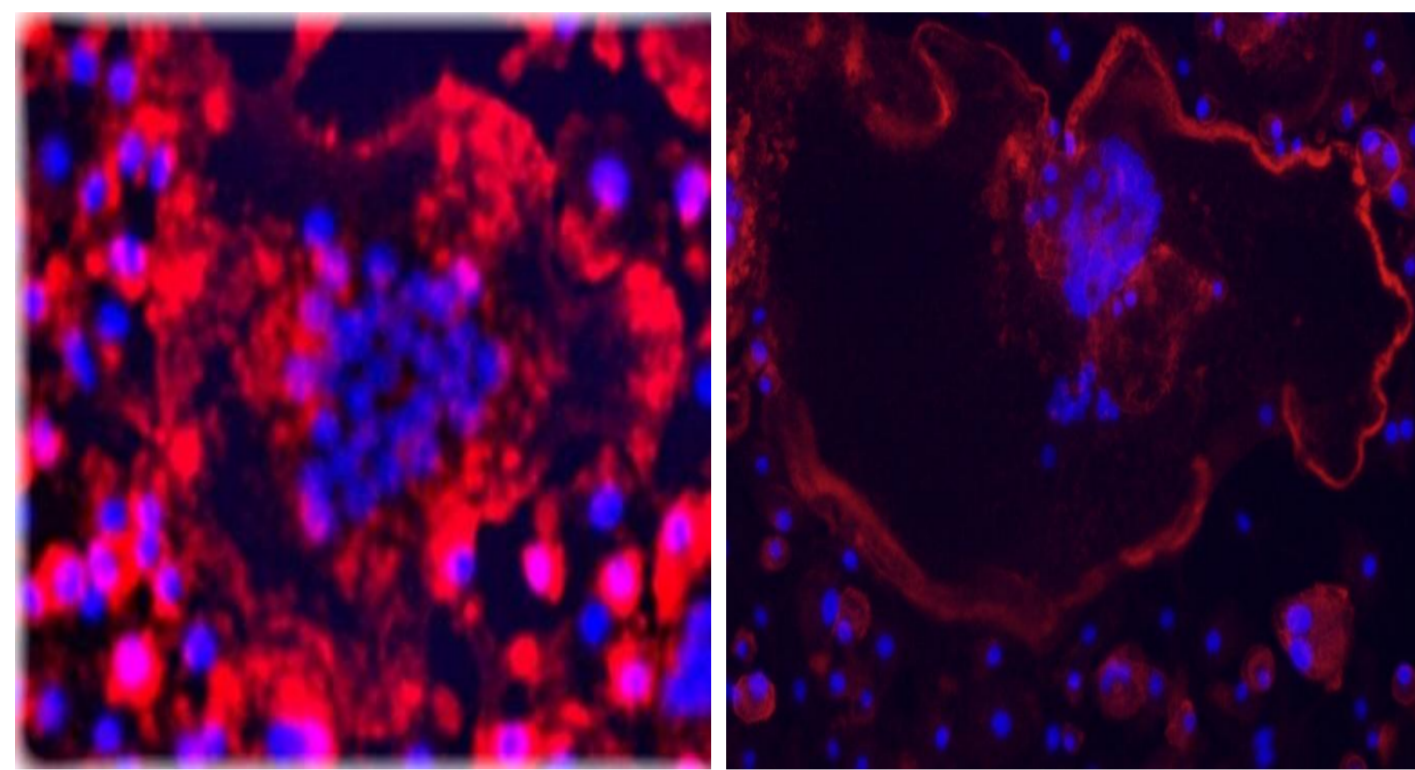


Fig. 1. DAPI and Phalloidin staining of MGCs. Image on the left shows characteristic osteoclast with multinuclei and F actin rings while the image on the right shows FBGC which also form F actin rings though not as complete as osteoclasts.

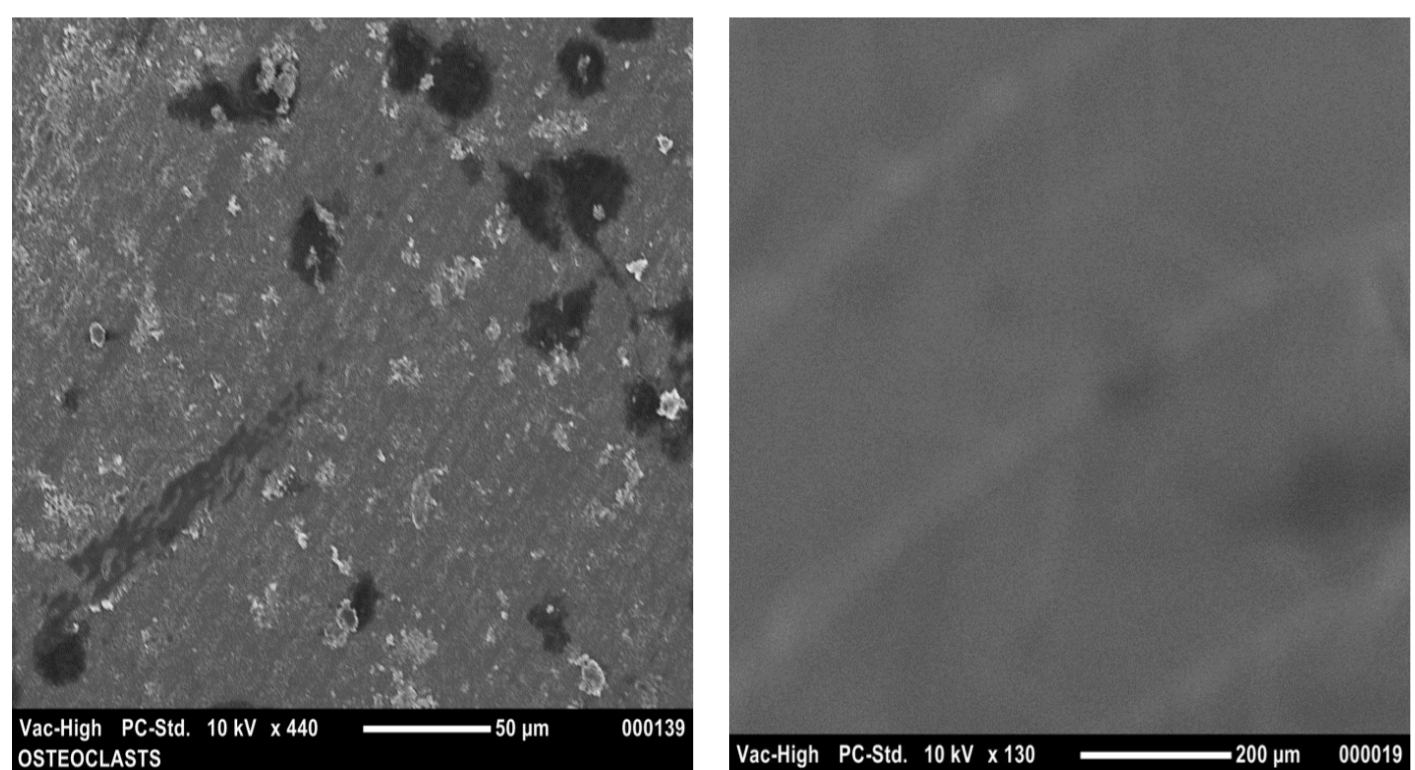


Fig. 2. Bone resorption assay. SEM image on the left shows bone resorption pits with osteoclasts while FBGCs on the right don't resorb bone.

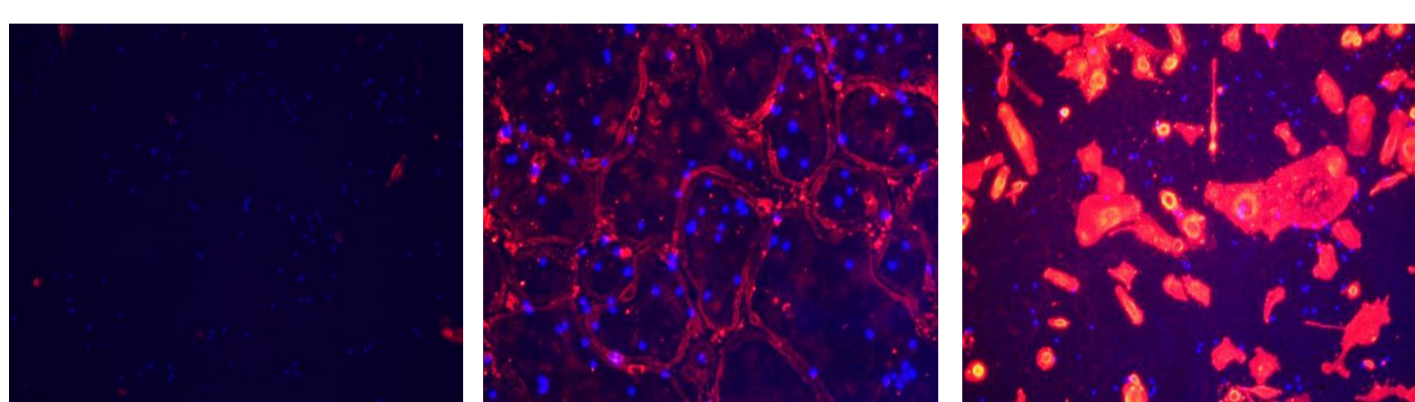


Fig. 3. Immunofluorescence of MGCs with OC-STAMP antibody. Monocytes (Left) did not show any OC-STAMP while osteoclasts (Centre) and FBGCs (Right) were both positive.

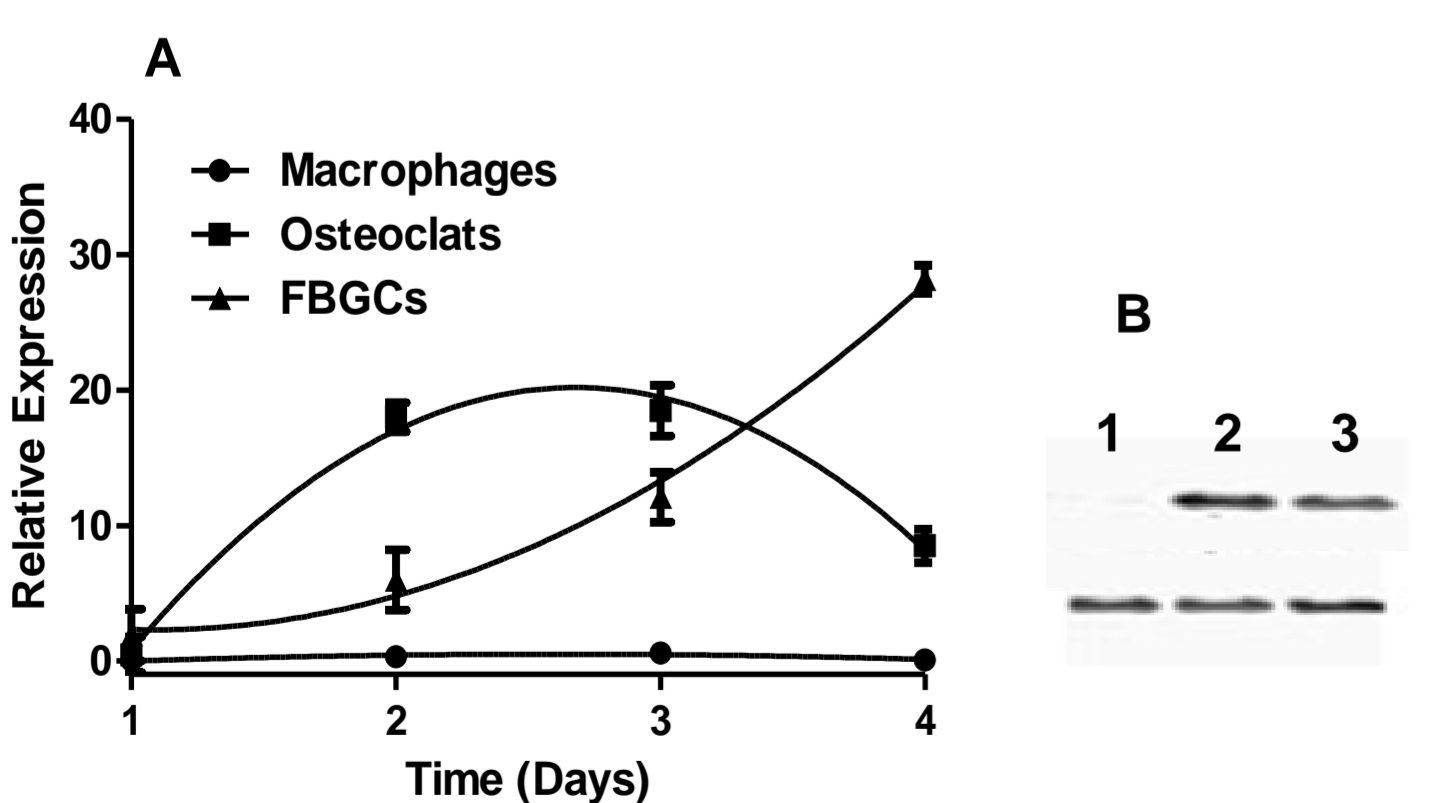


Fig. 4. OC-STAMP expression in MGCs. A. Osteoclasts and FBGC expressed more OC-STAMP than macrophages. On day 4 FBGCs expressed oc-stamp three folds more than osteoclasts. B. Western blot shows that monocytes don't express OC-STAMP while osteoclasts and FBGCs express the same amount (first line). Second row loading control calnexin.

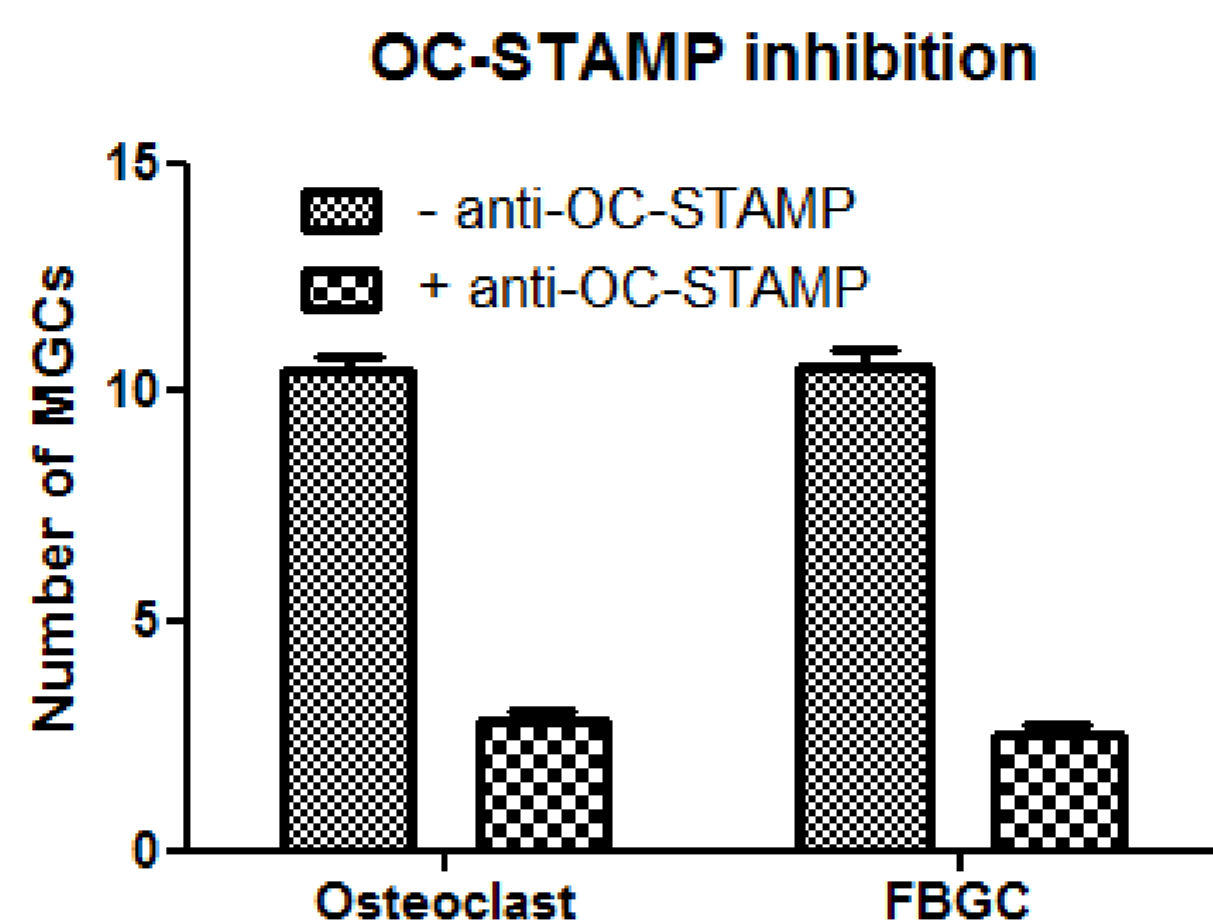


Fig. 5. TRAP positive multinucleated cells after treatment with anti-OC-STAMP antibody. There is significant difference between osteoclasts and anti-OC-STAMP inhibited osteoclasts. The same effect can be seen in FBGCs inhibited with anti-OC-STAMP antibody.

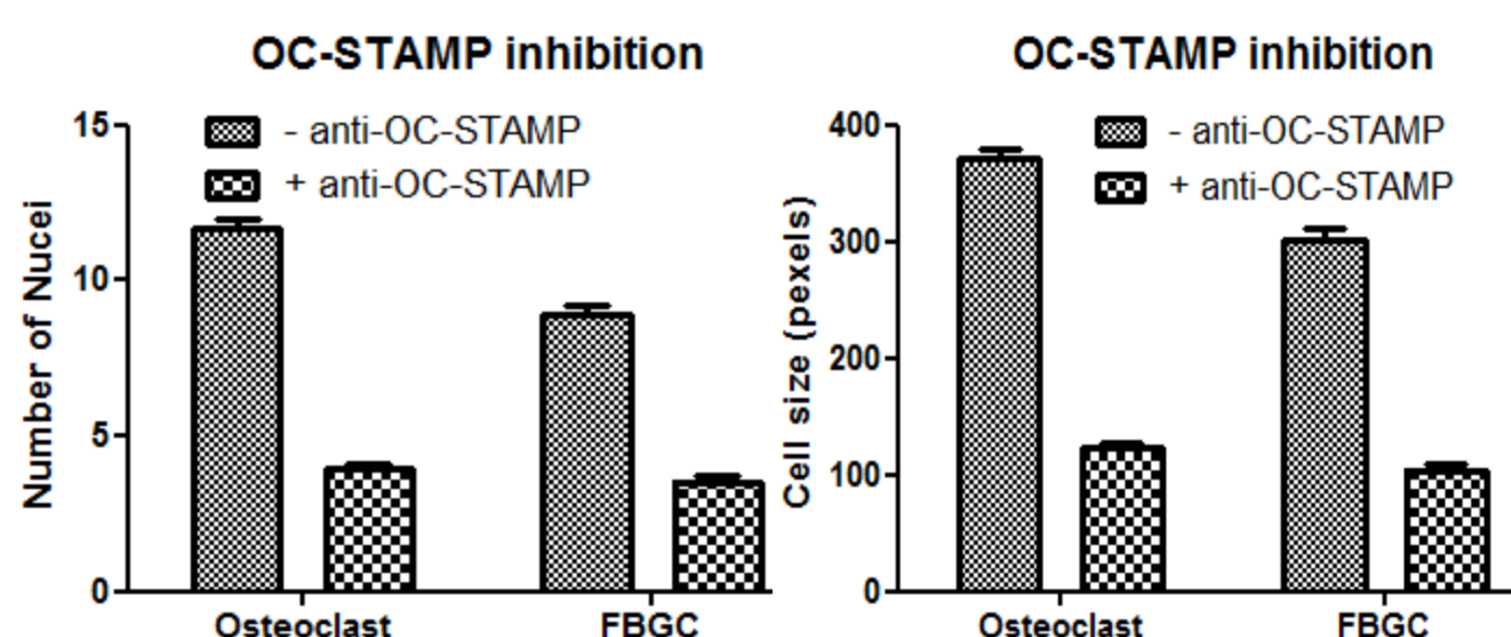


Fig. 6. Nuclei count and cell size in normal MGCs and OC-STAMP inhibited MGCs. Left graph, number of nuclei per Multinucleated giant cells. Anti-OC-STAMP antibody reduced the number of nuclei in both the FBGCs and Osteoclasts. Right graph, size of the multinucleated cells. Anti-OC-STAMP antibody reduced the fusion of monocytes and caused reduction in size of osteoclasts and FBGCs.

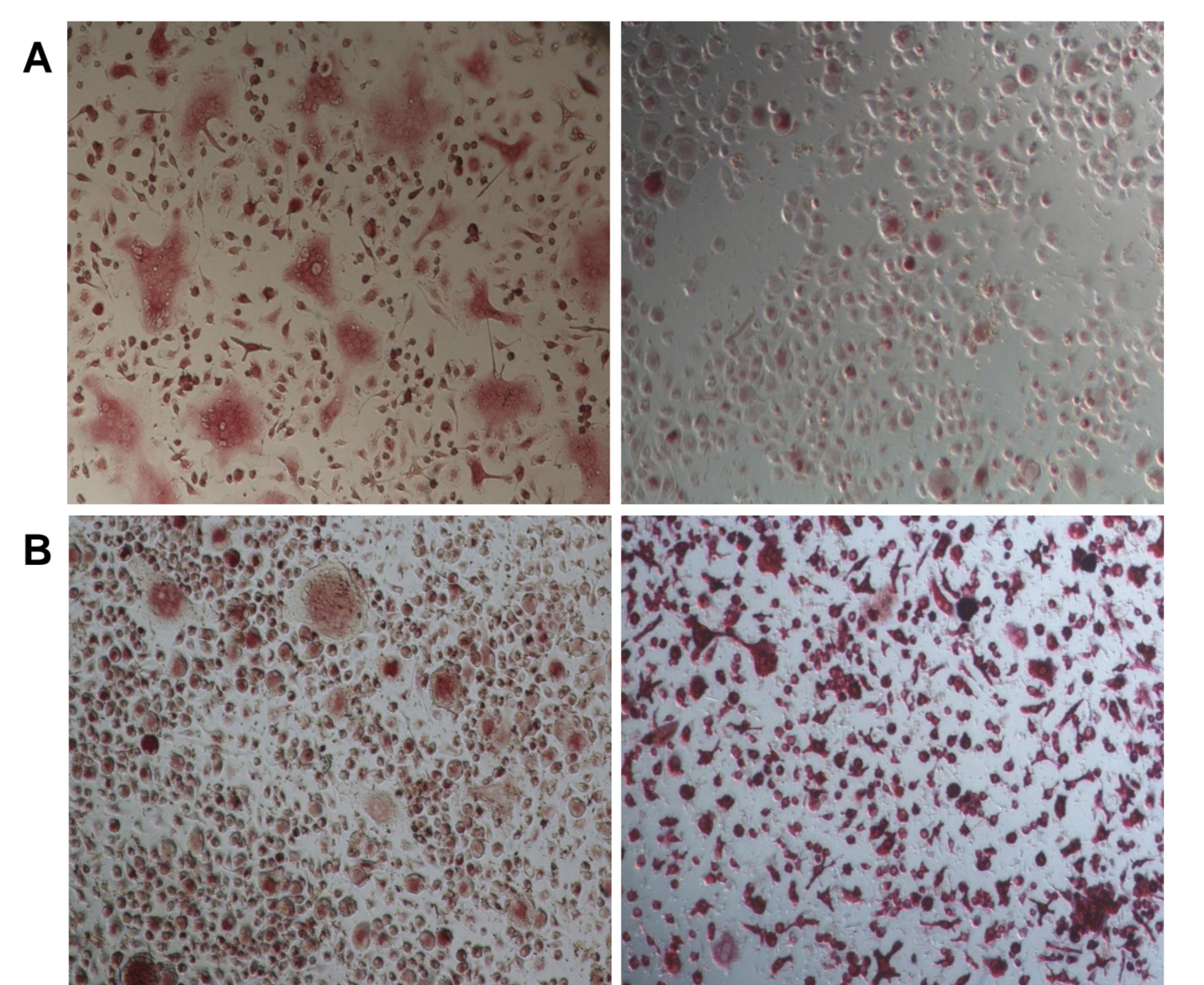


Fig. 7. TRAP staining for osteoclasts and FBGCs. A. Osteoclasts can be seen as multinucleated giant cells while on the second picture OC-STAMP antibody was added which inhibited the formation of osteoclasts. B. Foreign body giant cells can be seen as multinucleated giant cells while in the second picture OC-STAMP antibody was added which prevented the formation of FBGCs.

Discussion and Significance: Fusion of macrophages leads to FBGCs and osteoclast formation, both of these cells can be found in close proximity during peri implantitis (10). FBGCs found near rejected implant is reported to be TRAP positive (11) while those that are generated during in vitro culture on the other hand were observed to be TRAP negative (12).

Here we have show that when bone marrow macrophages were cultured for four days, they remained TRAP negative contrary to osteoclasts that were TRAP positive on day four, but when the culture was extended to day eight TRAP positive FBGCs were noted. On day 8 FBGCs developed the F actin ring which is characteristically found in osteoclasts (13). IL-4, that induces foreign body giant cells, is reported to suppress RANKL mediated TRAP expression but also increases the TRAP expression on its own (14). IL-4 is considered a switch that directs the bone marrow cells toward FBGCs rather than osteoclasts. When these giant cells were plated on bone they failed to resorb compared to osteoclasts, showing that they lack the capacity to resorb bone.

OC-STAMP is identified as one of the RANKL induced protein during osteoclast formation. Here we show the involvement of OC-STAMP in FBGCs formation. OC-STAMP has a functional and structural similarity to DC-STAMP which is important for both osteoclasts and FBGCs, so we tested OC-STAMP in both these cells and found out that not only it is expressed at RNA and protein level but inhibition of OC-STAMP leads to decrease in fusion in both osteoclasts and FBGCs.

Conclusion:

1. OC-STAMP is important for both Osteoclast and FBGCs
2. OC-STAMP inhibition leads to attenuation of fusion
3. FBGCs and osteoclast are different cells and FBGCs does not resorb bone

Acknowledgement:

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