Delivery of rapamycin using in situ forming implants induces immunoregulatory mechanisms promoting vascularized composite allograft survival.

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Introduction
Unlike solid organ transplantation, VCA offers unique opportunities for local delivery of immunosuppressive agents directly to the graft. Drugs administered directly into the graft may not only reduce potential side effects but also directly influence the magnitude and nature of an allogeneic immune response by promoting immune-regulation and tolerance through the expansion of donor-specific regulatory T cells (Treg).

In this study, we developed an in situ forming implant (ISFI) loaded with rapamycin to promote acceptance of vascularized composite allotransplantation (VCA) by inducing an immunoregulatory microenvironment.

Material / Methods
Development of in situ forming implant (ISFI) and evaluation in vitro and in vivo prior to application in experimental setting

Experimental setup

Results
Rapamycin-loaded ISFI promote survival of VCA

Rapamycin treatment promotes multilineage mixed chimerism. Chimerism of both lymphoid and myeloid lineages was significantly higher in all rats treated with Rapa-ISFI at POD21. At this time, the levels of myeloid chimerism were elevated and positively correlated with graft survival, suggesting that initial high levels of donor granulocytes and monocytes may correlate with the engraftment of donor pluripotent hematopoietic stem cells (HSC). Importantly, donor T cell levels, although low at the beginning of treatment, increased with time, reaching the highest values at the endpoint in all rapamycin-treated groups, further confirming the capacity of rapamycin to promote engraftment of donor HSC and therefore graft survival.

When compared to group 1, rats of Group 2 had significantly higher frequency of Treg in the peripheral blood (p=0.044), rats of Group 3 also had higher frequency of Treg, but it did not reach statistical significance (p=0.145) and rats of Group 4 had unchanged Treg frequency. Notably, the injection of Rapa-ISFI on the transplanted side promoted the expansion of Helios

Conclusion
Our study shows that low-dose delivery of rapamycin by ISFI successfully promotes immunoregulation inducing mixed chimerism and donor-specific Treg that in turn facilitates establishment of peripheral tolerance and long-term acceptance of VCA. Rapa-ISFI therapy represents a promising approach for decreasing toxicity, increasing patient compliance and, importantly, favoring the reprogramming of allogeneic response toward regulatory function.