Bench-Top Assay of Inflammation with Human Macrophages on Biomaterials

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Statement of Purpose: The foreign body response remains a powerful force that determines the success or failure of biomaterials. The gold standard of biocompatibility tests assess safety and efficacy of biomaterials using subcutaneous implantation in animals, which are expensive, time-consuming, and potentially a poor model of the human immune system (Seok, PNAS 2013,110:3507). Several recent studies have suggested *in vitro* behavior of human macrophages may predict clinical outcomes (Brown, *Acta Biomat* 2013, 9:4948). In particular, the pro-inflammatory (M1) vs. anti-inflammatory (M2) behavior is typically compared between biomaterials, with promotion of M2 behavior suggested as a desired outcome. However, the term, M2 macrophages, oversimplifies their behavior, which is more accurately described as subtypes that have different and distinct roles. M2a and M2c macrophages promote tissue deposition/fibrosis and remodeling, respectively (Spiller, *Biomater* 2014, 35:4477). Furthermore, macrophages are known to exist *in vivo* as hybrid phenotypes, exhibiting characteristics of M1, M2a, and M2c. Thus, there is a need to determine the influence of the different macrophage phenotypes in response to biomaterials, and whether or not their behavior *in vitro* can be used to predict clinical outcome. In this study, we explored the response of primary and THP1-derived human macrophages to four biomaterials commonly used for wound healing in humans.

Methods: Primary human monocytes and THP1 cells were cultured with 20ng/mL MCSF or 320nM PMA, respectively, to differentiate the monocytes into M0 macrophages. M0 macrophages were polarized into controls using previously described methods (Spiller 2014). Cells ($9.5x10^5$/scaffold) were cultured on 5mm biopsy punches of INTEGRA® Dermal Regeneration Template (Integra), PriMatrix® Demal Repair Scaffold (PriMatrix), AlloMend® Acellular Dermal Matrix (AlloMend), and OASIS® Wound Matrix (Oasis) for one hour before adding culture medium. RNA was extracted from the samples after 2 or 6 days (media change on day 3) and processed for RT-PCR. Expression of all genes were normalized first to GAPDH and then to the M0 control ($2^{-\Delta\Delta Ct}$). Data is represented as mean ± SEM (n=5). Statistical analysis between time points was performed using a two-way ANOVA and Bonferroni post-hoc test; analysis within a time point was performed with a one-way ANOVA with Tukey's post-hoc test.

Results: Culture of primary cells onto the scaffolds over time (comparing expression from Day 2 to Day 6) indicated that Oasis and PriMatrix promoted upregulation of TNFα and IL1β, respectively; Integra promoted down regulation of MDC (data not shown) and TIMP3, while PriMatrix and AlloMend promoted an upregulation of CD163. Differences within time points were also observed; Oasis promoted upregulation of TNFα compared to M0 Control, Integra and PriMatrix on Day 6. All scaffolds downregulated TIMP3 compared to the M0 Control on Day 6. Integra promoted upregulation of CD163 on Day 2 compared to M0 Control, PriMatrix and AlloMend, while AlloMend promoted upregulation of CD163 compared to M0 Control, Integra and Oasis on Day 6 (Fig. 1). THP1 macrophages on scaffolds exhibited significantly different TIMP3 and CD163 expression compared to primary macrophages (data not shown).

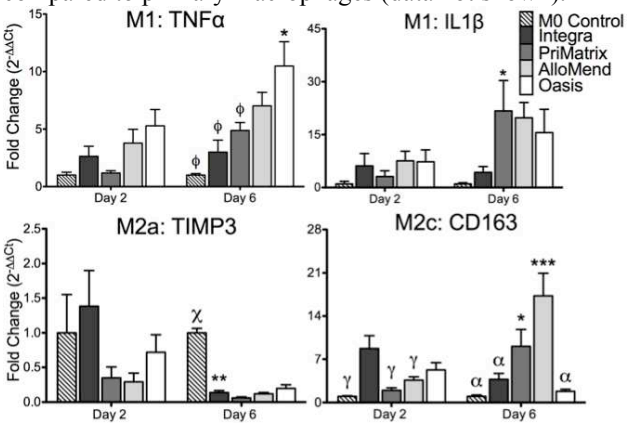

Fig. 1 - Gene expression analysis of primary macrophages on scaffolds over time. * indicates $p<0.05$ relative to the same group at Day 2 (**$p<0.01$, ***$p<0.001$). Comparisons within a time point (sample v. sample) are represented with γ ($p<0.05$ vs. Integra), α ($p<0.05$ vs. AlloMend), ϕ ($p<0.05$ vs. Oasis), and χ ($p<0.001$ vs. all other samples).

Discussion: Integra promoted an anti-inflammatory (low TNFα and IL1β), anti-fibrotic (downregulated TIMP3 and MDC) over time, and early pro-remodeling/matrix degradation (upregulated CD163 on Day 2) macrophage response, indicating the potential for Integra to integrate into the wound early and suppress scar formation, which has been observed clinically (Moimenn, *Plas Recon Surg* 2006:117(7S), p.989). Porcine small intestine submucosa (Oasis) has been associated with severe chronic imflammatory responses in abdominal hernia repair in rats (Bras, LE *Hernia* 2011:16(1), p.77), which was observed in this study by upregulated TNFα over time and compared to M0 Control, Integra, and PriMatrix on Day 6. PriMatrix exhibited pro-inflammatory/pro-angiogenic (upregulated IL1β) and pro-remodeling response (upregulated CD163) over time, suggesting a role in neo-vascularization (Spiller 2014; Rennert, RC *Int J of Biomats* 2013:3).

Conclusions: Primary human macrophages cells cultured onto biomaterials detect subtle differences in M1, M2a and M2c macrophage behavior that suggest differences in clinical outcome. These results highlight the potential to use gene expression of multiple markers of macrophage phenotype in a benchtop assay of inflammation.

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