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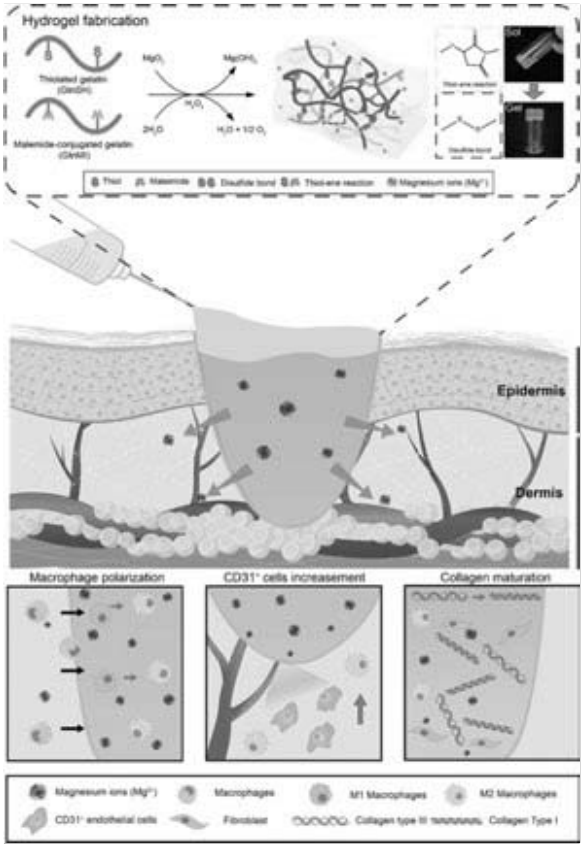
healing by promoting Mg^{2+}-induced M2 macrophage polarization and neovascularization *in vivo*. Therefore, we expect that our Mg-Gels can serve as bioactive matrices for tissue regeneration through easy fabrication, long-term Mg^{2+} encapsulation, and bioactivities.



119 - In Vitro Modulation Of Macrophage Phenotype By 3D Custom-made Micro Scaffolds

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***Purpose/Objectives:** Upon *in vivo* implantation of a microstructured device, a complex physiological process called foreign body reaction (FBR) takes place, characterized by a cascade of events involving many key players. An essential role is carried out by immune cells, specifically macrophages, that can polarize towards the M1 (pro-inflammatory) or M2 (pro-healing) phenotype. Optimizing 3D micro scaffolds design for prompting an anti-inflammatory response of macrophages is a fundamental strategy for achieving successful implant integration and tissue regeneration *in vivo.*

***Methodology:** 3D micro scaffolds were fabricated by 2-photon polymerization of the SZ2080 biocompatible photosensitive resin. Different pore sizes were tested for their ability to polarize mouse macrophages, ranging from 10x10x15 μm^3 to 50x50x20 μm^3. M0 macrophages were stimulated with 1µg/mL *Escherichia coli* lipopolysaccharide (M1, pro-inflammatory) or 40 ng/mL interleukin-4 (M2, anti-inflammatory) for 24 hours. Their polarization was evaluated after 48 h, in the presence of the micro scaffolds and on a flat glass surface, by confocal laser scanning microscopy (CLSM) and Western blot, quantifying the expression of specific biomarkers (inducible nitric oxide synthase, iNOS, and Arginase 1, Arg1). These analyses enabled us to determine which micro scaffold and to what extent contributed to pushing macrophages toward an M2 pro-healing phenotype.

***Results:** The *in vitro* experiments allowed us to evaluate the contribution of the 3D micro scaffolds' and their different pore sizes in directing the polarization of macrophages. Quantification of protein expression levels by immunofluorescence and Western blot showed that the presence of micro scaffolds does not promote M1 polarization (iNOS expression), while to favor an M2 pro-healing phenotype, it is necessary to choose larger pore scaffolds, such as 50x50x20 μm^3. Moreover, the presence of the micro scaffolds showed a significant increase in Arginase 1 expression, maximizing macrophage polarization toward M2.

***Conclusion/Significance:** The experiments showed that different 3D micro scaffold geometries can influence macrophage behavior, favoring their polarization to a pro-inflammatory or pro-healing phenotype and confirming their role in determining macrophage response. This finding suggests that the design of microstructures for decorating devices used for *in vivo* implantation needs to consider specific tridimensional features for preventing fibrotic encapsulation and favoring a successful integration. An in-depth analysis of the role of 3D micro scaffolds in influencing rearrangements in the macrophage cytoskeleton with activation of mechanotransduction pathways will be performed, together with an evaluation of specific bioactive molecules released into cell culture supernatants.

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120 - Liver-targeting Microparticles For Hepatocyte Transplantation

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***Purpose/Objectives:** Hepatocyte engraftment is a critical challenge for liver cell therapies. The majority of transplanted cells are cleared from the liver by proinflammatory cytokines, chemokines or receptors following instant blood-mediated inflammatory reaction or after the activation of resident macrophages. The addition of immunomodulatory cytokines, such as IL-10, or etanercept, a TNF blocker which specifically binds to TNF-α, has potential to reduce inflammation and improve cell transplantation. Encapsulation of these molecules within microparticles (MPs) for co-transplantation with hepatocytes provides controlled release strategies to increase cell engraftment. However, MPs typically fabricated with commercially available polymers are non-specific to the liver, resulting in inefficient delivery of encapsulated molecules. The asialoglycoprotein receptor (ASGPR) exhibits high affinity as a galactose receptor and is the only liver-specific receptor identified on hepatocytes. We demonstrate targeted drug delivery to the liver via microparticles fabricated with galactose ligands to achieve targeted release profiles. Using suitable mice model, co-transplantation of MPs and hepatocyte can promote hepatocyte survival.

***Methodology:** Galactosylation of poly(lactic-co-glycolic acid) (Gal-PLGA) was synthesised in-house. In vitro functional release was measured using ELISAs. Immunomodulatory response of MPs in vitro was performed using THP-1 differentiated macrophages. In vitro MP-cell attachment was performed using HepG2. In vivo biodistribution was performed using wild type mice through hepatic portal vein and intrasplenic injections. Evaluation of in vivo response to IL-10 and etanercept loaded microparticles was performed using CCl4 and $Ah^{Cre}Mdm2^{fl/fl}$ mice models that induce liver injury and injury + senescence respectively.

***Results:** MPs fabricated from Gal-PLGA demonstrated an enhanced MP retention (>85%, compared to 40% with conventional