Increasing Human Mesenchymal Stem Cell Attachment to poly(glycerol dodecanedioate), a Biodegradable Shape Memory Polymer

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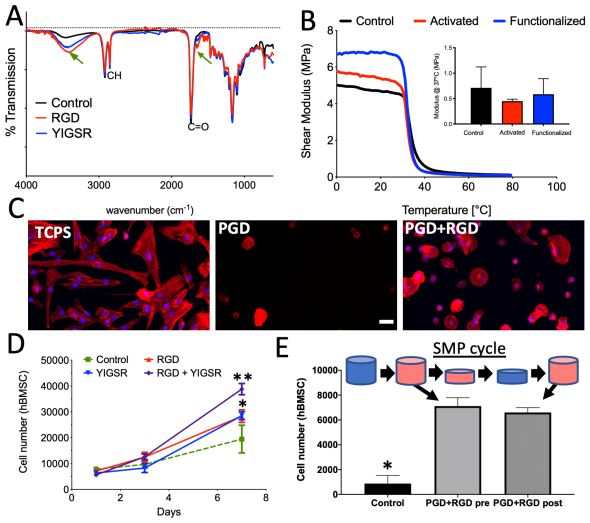
Statement of Purpose: Biodegradable shape memory polymers(bSMPs) are a growing family of materials primarily motivated by use in minimally invasive surgical(MIS) procedures[1]. These bSMPs are compressed during MIS delivery and expand *in vivo* when heated to body temperature(37ºC). We developed a bSMP[2,3], poly(glycerol dodecanedioate) (PGD) and composites[4], demonstrating tunable transition temperatures (T_{trans}) and non-linear elastic properties (at 37ºC). Although others have functionalized bSMPs to improve bioactivity[1], post-recovery bioactivity and selectivity of peptide grafted substrates remains to be investigated. Bone marrow stroma derived human mesenchymal stem cells(hBMSC) are of increasing clinical interest for regenerative medicine and immunomodulation. Laminin and fibronectin derived peptides known to target integrin receptors on hBMSCs were used to functionalize bSMPs. The primary aims of this study are 1) develop a process for functionalizing the surface of SMEs with cell adhesive peptides 2) assess biocompatibility of SMEs grafted with cell adhesive sequences 3) evaluate adhesive peptide functionality post-shape recovery.

Methods: PGD pre-polymer, a 1:1 molar ratio of glycerol and dodecanoate, was synthesized at 120ºC under N_2 for 24 hours followed by 3 mTorr vacuum for 24hrs. Pre-polymer was poured as flat sheets for an additional 38hrs at 120ºC and 90mtorr and sample discs were laser cut. Functionalization was performed by 1)activating surface through acidification/alkylation 2) forming isoacylurea intermediates with EDC/NHS 3) binding fibronectin derived GGGRGDSP peptide or laminin derived GGGYIGSR peptides. ATR-FTIR spectra were collected using a Shimadzu Prestige 21 Infrared Spectrometer to confirm functionalization. Shear moduli(8mm diameter x 1 mm thickness discs, n=3) were calculated using a temp sweep (-10ºC – 80ºC) on an oscillatory rheometer (Anton Paar MCR 302) at 1% strain and 5 rads/s. Human bone marrow derived mesenchymal stem cells(hBMSCs) were cultured on peptide coated substrates and controls (n=4) for 0-12 hrs to study initial attachment and for 7 days to study proliferation as previously described[5,6]. Finally, functionalized thin substrates(n=4) were heated, programmed, cooled to fix the shape and reheated to recover the permanent shape. Cells were added to SMPs upon recovery to investigate shape memory cycle effect on cell attachment. Statistical analysis was conducted using one and two-way ANOVA with Tukey pairwise comparisons in JMP.

Results: After validating processing conditions surface activation by exposing hydroxyl groups and binding of isoacylurea intermediates (data not shown), peptide groups were successfully grafted to PGD surfaces. Presence of peaks at 3400 cm^{-1}(N-H) and 1550 cm^{-1}(N-H, Amide II) were used to verify covalent binding of peptides to the material surface(Fig1a, green arrows). Temperature sweeps and rheometry indicate no significant differences across control, activated and functionalized samples T_{trans}(35.5 ±1.65ºC).

Figure 1. A) ATR-FTIR spectra of peptide functionalized PGD surfaces B) Rheometry temperature sweeps with inset moduli > 37ºC C) hBMSC after 12 hr on serum and serum free PGD and PGD=RGD substrates; scale bars 10 μm D) Cell proliferation on functionalized substrates , E) Cell attachment(3hr) on control, pre-programmed and post-recovered PGD+RGD substrates.



Furthermore, there were no differences in shear moduli at 37ºC amongst control, activated and functionalized samples indicating preservation of mechanical properties post-transition (Fig.1B). Taken together, these data verify that shape memory transition and the mechanical properties at 37ºC is largely unaffected by the functionalization process. MSCs cultured on GGGRGDSP grafted substrates demonstrated better cell attachment and spreading compared to control PGD substrates indicating preservation of peptide functionality (Fig1C). Additionally, hBMSC cultured on peptide coated PGD demonstrated increased proliferation compared to controls on day 7(Fig 1d. *p<0.05, ** P<0.001) suggesting greater attachment. Finally, peptide coated SMP substrates that underwent a shape memory cycle revealed greater cell attachment than PGD controls (Fig. 1e p<0.001). This is a significant finding of this study suggesting the preservation of peptide functionality when delivered percutaneously warranting further investigation *in vivo*.

Conclusions: This study provides both a framework for functionalization of bSMPs while preserving the shape memory effect and mechanical properties for use in MIS procedures for soft tissue repair. Future studies investigating the conservation of bSMP grafted peptide functionality *in vivo* and integrin selectivity are underway

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