Injectable and inherently vascularizing SIPN for delivering cells to the subcutaneous space

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Introduction: Injectable hydrogels are suitable to locally deliver therapeutic cells but lack of vascularization remains a limiting factor. Previously we have demonstrated that biomaterials containing methacrylic acid induced vascularization[1-3]. Here we report on an injectable semi-interpenetrating polymer network (SIPN) that can be used to subcutaneously deliver cells while promoting the formation of a perfusable vascular network at the implantation site.

Methods: As shown in **Figure 1**, injectable SIPN were obtainable by reacting a blend of 8-arm vinyl sulfone terminated polyethylene glycol (PEG-VS) and sodium polymethacrylate (PMAA-Na) with a stoichiometric amount of dithiothreitol (DTT). Two SIPNs were prepared by changing the PMAA-Na molar feed: PP8020 contained 80 % mol ethylene glycol and 20 % mol sodium methacrylate. PP9010 contained 90% mol ethylene glycol and 10 % mol sodium methacrylate. The hydrogels were characterized in terms of gelation time, swelling, stability in aqueous media, and stiffness. The vascular regenerative effect was investigated upon subcutaneous injection into CD1 mice.

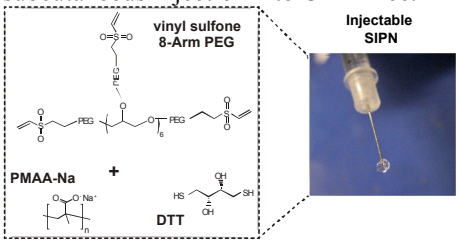
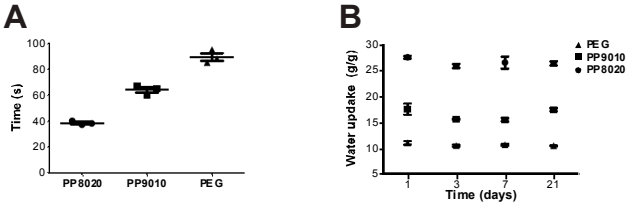


Figure 1: Injectable SIPNs were prepared by reacting PEG-VS, PMAA-NA, and DTT at physiological temperature, osmolality, and pH.

Results:

1. Physical Characterization: All the precursor solutions formed a solid 3D network in less than 2 min, with decreased gelation times with higher PMAA-Na molar feed in the formulation of SIPN (**Fig 2A**). The swelling in distilled water was highly sensitive towards the PMAA-Na molar feed (**Fig 2B**). Residual mass of > 95% were observed after 21 days of incubation, suggesting that PMAA-Na was tightly entangled and did not diffuse out of the SIPN (**Fig 2C**). Only slight differences in the stiffness were observed among the three formulations (**Fig 2D**).



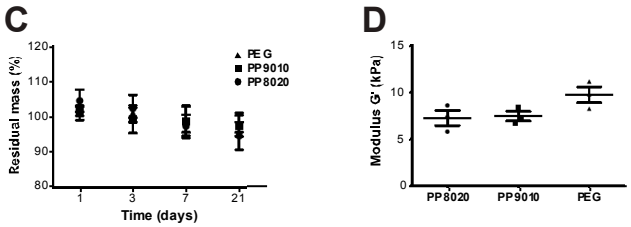


Figure 2: (A) Gelation times at 37°C as determined by the vial-tilting method. (B) The swelling and (C) residual mass upon incubation in distilled water. (D) The average frequency modulus (G'). PEG: PEG hydrogel without PMAA-Na.

2. Vascular regenerative effect *in vivo*: Only CD1 mice treated with SIPN containing 80% mole of PMAA-Na (PP8020) had significantly increased vessel density at day 7 ($p < 0.0001$), day 14 ($p < 0.0001$), and day 21 ($p < 0.003$) (**Fig 3A**). The generated vessels were perfused as early as day 7 as evidenced by tail-vein injection of fluorescent-labeled lectin (**Fig 3B**).

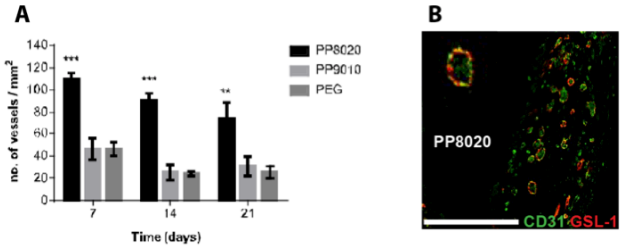


Figure 3: (A) Quantification of blood vessel density in the immediate vicinity of hydrogels. (B) Confocal image of histological sections at day 7 after tail-vein injection of fluorescent-labeled lectin. Scale = 200 µm

3. Cell delivery: Rat islets were used to investigate the suitability of SIPN as cell delivery vehicle. The *in vitro* viability of islets embedded in SIPN and secretion of insulin remained unchanged compared to free islets. Upon subcutaneous injection, insulin positive islets were present at day 7.

Conclusions:

Our approach establishes an effective biomaterial-mediated strategy to deliver cells while enhancing vascularization in the surrounding tissues.

References:

1. Lisovsky *et al*., Biomaterials 98 (2016) 203 – 214.
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3. Butler *et al*., J. Biomed. Mater. Res. Part A, 82 (2007). 265-273.