Plasma-based hydrogels for the treatment of deep-partial thickness burns

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Statement of Purpose: Current tissue engineered skin equivalents rely on thin dermal substitutes to allow for nutrient diffusion to increase cell viability and attachment. One strategy to overcome this limitation is to develop treatments that enhance vascularization within tissue engineered scaffolds *in situ*. Platelet-rich plasma (PRP) and/or platelet-free plasma (PFP) may provide an autologous matrix source, and is currently being used for clinical applications including articular resurfacing, tendon repair, and wound healing. We have previously shown that adding PEG (PEGylation) to pure fibrinogen before mixing with thrombin produces a unique hydrogel.[1] Herein we show that PFP can be used as a source of fibrinogen, and PEGylation leads to hydrogel-like characteristics rather than an amorphous fibrin clot. Furthermore, we demonstrate the feasibility of applying these biomaterials for burn wound healing in a porcine deep partial thickness model.

Methods: Fresh human PRP was provided by the Division of Hematology located at the USAISR (IRB#: H-10-023). To obtain PFP, PRP was centrifuged at 4,300xg for 30 minutes at 24ºC. PFP was PEGylated with PEG concentrations dictated by the amount of fibrinogen in the plasma[1]. PEG-PFP mixtures were then polymerized either by adding $CaCl_2$ (11mM to 30mM) or human thrombin (Th: 5U to 20U) and both rheology and SEM experiments were performed. Finally, *in vitro* results provided the selection criteria to test the ability of plasma hydrogels to improve wound healing in a porcine deep partial thickness burn model. Briefly, PEG-PFP hydrogels were prepared from whole pig blood as described above. Three cm diameter brass probes were heated to 100°C and applied for 27 seconds on the dorsum of anesthetized Yorkshire pigs. On post-injury day 4, wounds underwent surgical debridement and were treated with hydrogels using a dual syringe applicator *in situ*.

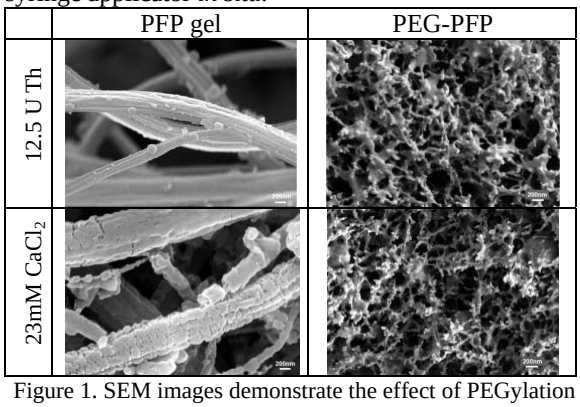

Figure 1. SEM images demonstrate the effect of PEGylation on gel formation. Regardless of clotting agent, the addition of PEG allows for a distinct structure with an elaborate porous network.

Results: Whether $CaCl_2$ or Th was used (Figure 1), PEGylation of PFP yielded viscoelastic hydrogels and increased pore size, while unPEGylated gels generated formation of normal fibrous clots, appeared opaque, and easily deformed when handled. Storage modulus values for PEG-PFP gels were 71.63 ± 9.2 and 87.43 ± 17.8Pa at 23 and 27mM $CaCl_2$, respectively. Storage modulus values resulting from gels made with Th generated a storage modulus of 47.3 ± 4.6, 73.55 ± 11.7, and 92.58 ± 19.2 Pa for 5U, 10U and 12.5 U, respectively, which is very similar to values seen using pure fibrinogen (~92Pa). Increasing the concentration of both Th and $CaCl_2$ led to decreases in pore size and increases in pore density as seen via SEM. Moreover, increasing Th concentrations decreased the time needed for hydrogels to form. *In vivo,* PEG-PFP mixture polymerized with Th successfully formed stable hydrogels within a short time period (~35 seconds). These hydrogels adhered to the contours of debrided burn wounds (See Figure 2) and integrated into the wound beds.

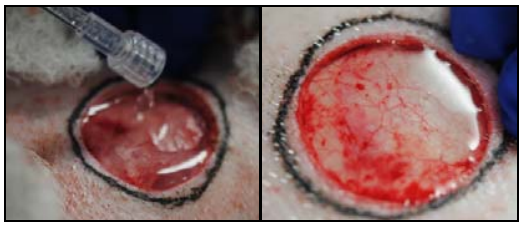

Figure 2. Left panel shows *in situ* formation of gels from a dual syringe applicator. After application, stable hydrogels were formed within 35 seconds (right panel).

Conclusions: We have demonstrated that plasma can be PEGylated to generate a stable, viscoelastic and easy to handle hydrogel. Using $CaCl_2$ or Th as polymerizing agents led to differences in viscoelastic properties and gel pore size. Applying these hydrogels *in situ* will elucidate whether or not PFP hydrogels accelerate and/or enhance wound healing compared to other treatments. This model can be used with or without debridement as a platform for testing biomaterial and stem cell combinations in order to improve wound healing[2]. The use of PFP/PRP will provide an alternative autologous matrix and create a cost effective wound coverage in order to accelerate wound healing. Addition of the appropriate cells to these hydrogels may overcome the shortcomings of other currently used clinical skin substitutes or grafts.

References:

[1] Natesan S et al. Tissue Eng PartA. Apr 2011: 17(7-8), 941-53.
[2] Singer et al. J Burn Care Res. 36(6), 647, 2011.

Acknowledgments

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Disclosures

This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.