Interpenetrating Networks Containing Gelatin Modified with PEGylated RGD and Soluble KGF: Synthesis, Characterization, Application in *in vivo* Critical Dermal Wound

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Statement of Purpose: The addition of functional peptide sequences and/or soluble factors to biomaterials is an active field of research in promoting material biocompatibility and efficacy in mediating cellular/tissue responses. We have developed a binary *in situ* photopolymerizable interpenetrating network (IPN) system composed of poly(ethylene glycol)(PEG)-ylated immobilized factors and soluble factors to complement the host response. Previous work has demonstrated the synthesis of this multifunctional IPN composed of PEG diacrylate (PEGdA) and chemically modified gelatin for use as tissue scaffolds and drug delivery vehicles. The purpose of this study was to evaluate the biocompatibility and the efficacy in wound healing of a gelatin-based IPN containing PEGylated RGD and soluble KGF-1.

Methods: IPNs were composed of PEGdA and either RGD-modified gelatin (RGD-IPN+KGF) or unmodified gelatin (unmod-IPN) at a 6:4 ratio. KGF-1 was added to the RGD modified gelatin solution at a concentration of 3.3 ng/ml which has been shown to stimulate keratinocyte proliferation. The method for the modification of gelatin with PEGylated-RGD follows previously established and characterized procedures. Intermediate products were characterized using a reverse phase HPLC system. The RGD-modified gelatin was characterized using a GPC system, ^{1}H-NMR, and a method based on trinitrobenzenesulfonic acid (TNBS) and spectrophotometry which quantifies the percent modification of gelatin lysyl groups. IPNs were applied to a full-thickness wound on Sprague Dawley rat models (n=3). Wound healing was assessed through histological grading of the host response and percent area contraction at 2 days, 1 week, 2 weeks, and 3 weeks. Unmod-IPN applied to a wound was also evaluated at 3 weeks. Sham controls were not feasible due to the high risk of infection and the massive trauma induced.

Results / Discussion: There was a significant difference in the amount of contraction between wounds to which RGD-IPN+KGF (11 ± 11 percent reduction in wound area) and unmod-IPN (34 ± 6.8 percent reduction in wound area) were applied during the first week ($p = 0.04$). At 3 weeks, wound contraction was similar for both groups where RGD-IPN+KGF wounds had an average of 100 ± 0.39 percent area reduction and unmod-IPN wounds had an average of 97 ± 3.8 percent area reduction. The progression of the inflammatory response was characterized over time through the amounts and types of cells present. Wounds treated with RGD-IPN+KGF progressed through a normal inflammatory response without re-eliciting injury or forming multinucleated cells. In general, unmod-IPN treated wounds had a higher inflammatory cell density than those wounds treated with RGD-IPN+KGF. Macrophage levels were significantly greater in the unmod-IPN (444 ± 209 cells/mm^2) as compared to the RGD-IPN+KGF (67 ± 15 cells/mm^2) ($p = 0.04$). Fibroblast levels were also significantly greater in unmod-IPN (1660 ± 771 versus 235 ± 38 cells/mm^2; $p = 0.03$). The extent of cellularity and organization of the extracellular matrix (ECM) was markedly higher for wounds healed with RGD-IPN+KGF than those healed with unmod-IPN. The resulting regenerated tissue treated with RGD-IPN+KGF demonstrated greater neovascularization and a basket-weave pattern in the ECM, especially towards the epidermis.

Conclusions: By including PEGylated-RGD and KGF-1 in the composition of the IPN, the healing of the wound progressed faster and the composition of the regenerated tissue was more favorable in structure as compared to wounds treated without those factors. These results indicate that both soluble and immobilized bioactive factors can be incorporated into our IPN platform to enhance the rate and the quality of dermal wound healing.

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		Cell Density (cells/mm^2)			
		Neutrophils	**Macrophages**	**Lymphocytes**	**Fibroblasts**
RGD-IPN+KGF					
Time post surgery	**Area of tissue**				
1 wk	**IPN-interface**	‡*2783 ± 793	*687 ± 378	*184 ± 52	*419 ± 63
2 wks	**IPN-interface**	*469 ± 577	235 ± 167	117 ± 88	956 ± 458
3 wks	**IPN-interface**	0 ± 0	⌂67 ± 15	25 ± 25	⌂235 ± 38
1 wk	**Undamaged**	0 ± 0	25 ± 0	42 ± 15	117 ± 52
2 wks	**Undamaged**	0 ± 0	59 ± 38	50 ± 44	268 ± 182
3 wks	**Undamaged**	0 ± 0	34 ± 38	25 ± 0	235 ± 102
Unmod-IPN					
Time post surgery	**Area of tissue**				
3 wks	**IPN-interface**	0 ± 0	*444 ± 209	151 ± 91	*1660 ± 771
3 wks	**Undamaged**	0 ± 0	67 ± 73	17 ± 29	268 ± 139

* Significantly different from undamaged tissue ($p<0.05$); ‡ Sig. different from 2 weeks RGD-IPN+KGF interface ($p<0.05$); † Sig. different from 3 weeks RGD-IPN+KGF interface ($p<0.05$); ⌂ Sig. different from 3 weeks unmod-IPN interface ($p< .05$)