Multifunctional Biomaterial Matrix for Advanced Wound Healing

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Background: Modern wound dressings provide a moist healing environment and facilitate faster and higher quality of healing. A new semi-interpenetrating network (sIPN) biomaterial platform based on poly(ethylene glycol) (PEG) and gelatin was developed as a multi-functional matrix for wound care.

The Problem: Besides providing a moist environment and facilitating the healing process, advanced wound dressings may be designed to serve as delivery matrices for drugs and therapeutic cells. New and effective treatments should also comply with clinical settings and be easy to use. No single treatment exists today that can fulfill all these requirements; however, advancement in multifunctional biomaterial design and development holds promise to fill this technology gap.

Basic/Clinical Science Advances: PEG + gelatin sIPN provides an ideal platform for fundamental research in cell-cell and cell-biomaterial interaction that is important in wound healing. The *in situ* forming ability of sIPN facilitates its use in large and irregular wounds with complex contours and crevices.

Clinical Care Relevance: Although various commercially available wound dressings have been produced, a low-cost, easy-to-use, and biofunctionalizable biomaterial that provides a moist environment and facilitates healing is still a target of active tissue regeneration research.

Conclusion: Extensive preclinical data support the use of *in situ* polymerized sIPN in advanced wound care.

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Submitted for publication August 3, 2011.

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Abbreviations and Acronyms

3D = three-dimensional

PEG = poly(ethylene glycol)

PEGdA = poly(ethylene glycol) diacrylate

sIPN = semi-interpenetrating network

BACKGROUND

AMONG MANY FUNCTIONS, the skin provides a critical barrier against the surrounding environment. Traditional wound dressings composed of cotton wool, gauze, or other bandaging provide limited protection from foreign infiltrates. Further, moisture management, ease of use, and compliance remain major challenges for traditional dressings. Modern dressings have been developed to create a moist environment to facilitate healing. Both natural and synthetic

materials are used to produce modern dressings. Synthetic materials typically have greater mechanical strength and product consistency. However, unlike natural materials, many synthetic materials lack the functionality to promote desirable biological response. Some modern dressings including skin substitutes may eventually replace the use of human skin grafts in certain wound types. These tissue equivalents may be combined with cultured human skin cells as cell-based therapies.¹

Cell-based skin grafts have demonstrated efficacy in treating certain chronic wounds and represent a rapidly growing sector in wound care research. Our lab has developed a new semi-interpenetrating network (sIPN) biomaterial platform that is composed of a synthetic polymer, poly(ethylene glycol) (PEG), and naturally derived biomolecule, gelatin. The incorporation of gelatin enhances cell/tissue responsiveness and biofunctionality of sIPN as well as controls material physical properties including biodegradation. This hydrogel-like scaffold can be adapted as matrices for both drug delivery and cell-based therapy. The sIPN is currently being developed for an initial clinical application based on its broad potential as a multifunctional platform for wound care.

TARGET ARTICLES

1. Kleinbeck KR, Faucher I, and Kao WJ: Multifunctional *in situ* photopolymerized semi-interpenetrating network system (sIPN) is an effective donor site dressing: a cross comparison study in a swine model. *J Burn Care Res* 2009; **30**: 37.
2. Faucher LD, Kleinbeck KR, and Kao WJ: Multifunctional photopolymerized semi-interpenetrating network (sIPN) system containing bupivacaine and silver sulfadiazine is an effective donor site treatment in a swine model. *J Burn Care Res* 2010; **31**: 137.
3. Chung A and Kao WJ: Fibroblasts regulate monocyte response to ECM-derived matrix: the effect on monocyte adhesion, and the expression of inflammation, matrix remodeling and growth factor proteins. *J Biomed Mater Res* 2009; **15**: 841.

CLINICAL PROBLEM ADDRESSED

Quality wound healing requires several elements including the removal of dead tissue, eradication and prevention of microbial infiltrate, absorption of exudates, and regeneration of healthy skin.² Advanced wound treatments should facilitate these healing requirements, also provide a moist wound environment, and prevent scar contracture for ideal healing progression and end-stage outcomes. The delivery of therapeutics to the wound site may also assist in addressing ancillary issues such as pain management. Effective wound treatments should also be designed to facilitate clinical acceptance and compliance. Although no single treatment exists today that can fulfill all these requirements, advancements in multifunc-

tional biomaterial design and development hold promise to fill this technology gap.

RELEVANT BASIC SCIENCE CONTEXT

Various materials such as hydrocolloids, alginates, and hydrogels derived from different base material sources have been explored as potential biomaterials for advanced wound therapy. Hydrogels exemplify multiple key properties for wound care. The ability to absorb and retain high water volume renders hydrogels well suited for treating dry and moderately exuding wounds.³ Hydrogels also reduce wound exposure to oxygen in the air, which may assist pain management. One disadvantage of hydrogels is that they typically have lower mechanical strengths, thus reducing their ease of use and handling. The combination of natural materials and synthetic materials in one single multi-component hydrogel formulation could improve mechanical properties and impart biofunctionality. Based on this hypothesis, our lab designed a hydrogel-based tissue scaffold composed of synthetic PEG polymer and naturally derived gelatin, which could act as a dressing in wound treatment.

Drug delivery from a given wound treatment has been identified as a valuable way to improve the quality of wound care.⁴ Drugs released from traditional wound dressings can be highly effective. However, the requirement for frequent dressing changes strains patient compliance and provides added opportunity for microbial infiltration. Using hydrogels may provide longer-term controlled release, thereby prolonging drug action. Drug release kinetics from hydrogel systems are highly dependent on material characteristics and the drug conjugation method. Detailed research is required to increase our understanding of drug release kinetics and material design.

Although the understanding of the molecular mechanism of wound healing has substantially advanced in the recent years, the translation of that knowledge into single-molecule therapeutics has been disappointing. Thus, the use of biomaterial-enabled cell-based therapy represents a rapidly developing approach in advanced wound healing research.⁵ Cell-based skin substitutes may facilitate wound healing progression by providing biochemical signals that are analogous to those produced in normal healthy skin. However, various commercially available skin substitutes exemplify some key real-world challenges. For example, tissue equivalents are often very expensive and laborious to manufacture. Skin substitutes that employ autolo-

gous cells typically elicit favorable treatment outcomes, but donor sites for many therapeutic cell types are often limited in volume. Additionally, long culture times for cell expansion and high costs limit clinical utility, particularly when treating large wound areas and in acute care. Skin substitutes containing allogeneic cells may resolve cell shortages. However, allogeneic cells introduce a greater risk of immunogenic rejection after application.⁶ Stem cells have shown improved efficacy in the treatment of various wound types. However, the precise cellular mechanisms of stem cells treatment in wound healing are still not fully understood. Further, the use of stem cells in human disease treatment is still controversial.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

The sIPN wound dressing is composed of synthetic PEG polymer and naturally derived gelatin.⁷ Gelatin is the hydrolyzed product of collagen, a natural extracellular protein and one major component of human skin. High molecular weight PEG was acrylated to form PEG-diacrylate (PEGdA). Gelatin and photoinitiator (Irgacure2959) were then added to PEGdA in an aqueous medium to form the sIPN solution. PEGdA was rapidly cross-linked by using long-wavelength UV light to form hydrogel at neutral pH, which entraps gelatin within the polymer matrix.⁸ Rapid polymerization (<3 min) allowed the viscous sIPN solution to be directly applied onto the wound bed and polymerized *in situ*. The photoinitiator used in the sIPN formulation has been identified as noncytotoxic to several cell lines and primary cells and has been shown to be biocompatible in several animal models as indicated by published literature and MSDS from the manufacturer.⁹ The process of *in situ* photopolymerization has been adapted for medical applications with increasing frequency in the recent years. For example, light-activated curing of resin-based composite materials has long been used in dental surgery. Drug delivery capability can be easily adapted to the sIPN system. Numerous drugs and biomolecules have been incorporated into the sIPN and explored. These include antimicrobials, local anesthetics, and growth factors. Additionally, skin cells such as keratinocytes and fibroblasts were directly mixed into the PEGdA and gelatin solution and polymerized *in situ*, thus forming a three-dimensional (3D) cell construct that can present cell-derived pro-healing factors to the wound bed. Although the sIPN has been identified as a potential scaffold for drug and cell delivery and could act as an ideal wound dressing, there still

exist several unique challenges that require particular attention. Drug loading into the sIPN is accomplished via physical incorporation. Thus, the physical stability of these entrapped drugs as well as the impact on the polymerization process are unique to the chemical identity of the specific drug. It is likely that several mechanisms are involved in how each sIPN component and the combination thereof promote tissue regeneration. For example, sIPN provides a moist healing environment and a protective barrier, sIPN functions as a temporary tissue scaffold, whereas the extracellular matrix component provides biochemical signals in regulating cellular processes. However, the precise mechanism of how each sIPN component modulates tissue regeneration is not completely elucidated.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

sIPN hydrogel as a biocompatible, biodegradable, *in situ* forming wound healing dressing, and drug delivery matrix

In situ polymerization increased the utility of sIPN for treating large and irregular wounds with complex contours and crevices. The physiochemical characteristics of sIPN were optimized via gelatin modification, and varying gelatin and PEGdA weight ratio.⁷ The use of PEG matrix increased sIPN mechanical strength and stability in aqueous environments, whereas gelatin increased sIPN elasticity and biodegradability. sIPN biodegradation was observed after 3 weeks of subcutaneous implantation in a rat model.¹⁰ Drug molecules were directly incorporated into the polymer solution or covalently conjugated to gelatin before polymerization, which provided control over loading density and release kinetics. Drugs and proteins such as silver sulfadiazine, bupivacaine, and keratinocyte growth factor were loaded, and the release kinetics and efficacy were characterized.^{11–13} Antibacterial efficacy of silver sulfadiazine loaded sIPN was quantified on cultured bacteria. The results showed that silver sulfadiazine loaded sIPN can be bactericidal to gram-positive and gram-negative strains which indicated that the drug incorporated in sIPN is able to retain full functionality. Concurrent delivery of drugs through sIPN demonstrated the utility of this technology to improve wound healing and to address co-morbidity.¹²

sIPN as a platform to study cell-cell and cell-material interaction

Skin regeneration is a complex process requiring the interaction of many different cell and tissue

types. The sIPN provided a unique platform to study the mechanism of cell-material interaction. The incorporation of extracellular matrix-derived components such as gelatin and collagen promotes cell-material interaction by increasing the number of potential sites for cell attachment and migration. This biofunctionality was further exploited through conjugation with integrin binding peptides, such as RGD and PHSRN, before incorporation in the sIPN. RGD-modified sIPN modulated monocyte adhesion and subsequent gene expression of inflammatory and matrix remodeling factors *in vitro*.¹⁴ Co-culture of monocytes with fibroblasts reduced monocyte adhesion onto the sIPN surface and modulated cytokine expression in monocytes, thus suggesting a dynamic monocytes response to the sIPN in the presence of fibroblasts.¹⁵ This co-culture model was employed in subsequent sIPN studies to help further elucidate the complex autocrine and paracrine relationships between fibroblasts, keratinocytes, and monocytes in a broader context of biomaterial-mediated host response and wound healing.¹⁶

Cross comparison study with other wound dressing products in swine model

The sIPN was applied alongside multiple commonly used clinical treatments including ActicoatTM, TisseelTM/Talfa, and XeroformTM in a swine partial thickness wound model.¹⁷ The inflammatory response and features of dermal extracellular matrix remodeling were comparable across all treatment types and time periods. Histological observation of skin biopsies showed that sIPN treatment elicited wound healing within 2 weeks without overt toxicity. Further, the sIPN elicited more complete epidermal coverage than Acticoat and Tisseel at intermediate time points. In another study, the delivery of bupivacaine and sliver sulfadiazine from the sIPN did not negatively affect the healing efficacy.¹⁸ The application of these drugs in conjunction with sIPN may help prevent infection and improve patient pain care.

sIPN as 3D cell encapsulation platform for cell-based wound healing therapy

Cell-based therapy is an active field of research in wound regeneration. An engineered skin substitute can mimic the natural secretion of cytokines, growth factors, and ECM proteins, which are known to facilitate tissue regeneration. Both keratinocytes and fibroblasts were successfully and easily encapsulated into sIPN system. The viability of entrapped cells was observed to the last several weeks *in vitro*, thus indicating the potential of using sIPN as a cell-based therapy platform (on-

going work). Another rapidly developing method of treating chronic wounds is the introduction of stem cells to the wound bed. This approach has been associated with enhanced angiogenesis, re-epithelialization, and healthy dermal remodeling with limited evidence of immunological rejection. In our lab, allogeneic bone marrow- and adipose tissue-derived mesenchymal stem cells were implanted in a swine partial thickness wound model to further probe the influence of stem cells on wound healing in conjunction with the sIPN. A significant impact on gross wound outcome was observed in the co-application of sIPN with stem cells (unpublished observations).

INNOVATION

The combination of PEG and gelatin provides a flexible platform for wound healing research and utility. The *in situ* forming ability of sIPN improves key challenges associated with traditional wound dressings such as dressing detachment, shape adjustment, maintaining a moist environment, and minimizing foreign infiltrates. The sIPN system is easily modified with drugs such as cytokines and growth factors to promote healing and address comorbidities. The solid powder form of both PEGdA and gelatin facilitates manufacturing, transport, and improves shelf-life. Thus, the *in situ* photopolymerized sIPN system has the potential as an easy-to-use product for clinical application. The sIPN system also has the potential to serve as a matrix for a tissue-engineered skin substitute that may become a powerful tool for cell-based therapy in wound healing.

SUMMARY ILLUSTRATION

(A) The *in situ* photopolymerized sIPN is based on a matrix of crosslinked PEG and physical entangled gelatin. Therapeutic cells, peptides, proteins, analgesics, antimicrobials, and immunocytokines as soluble or immobilized molecules can be directly incorporated into the sIPN system to influence cell-material interaction and to enhance clinical utility. (B) sIPN combined with tissue engineering and cell-based approaches: keratinocytes and fibroblasts are encapsulated in the sIPN and form organogenic 3D skin substitutes that can be directly applied to the wound site. (C) Live/dead staining (upper image, green dots as live cells and red dots as dead cells, 10×) and H&E staining (lower image, blue as hydroporel, dark blue as nuclear and purple as cytoplasm, 100×) results showed that fibroblasts could maintain their viability after being encapsulated in the sIPN for 2 weeks.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Significant advances in material engineering and proofs of concept in preclinical studies have been made with sIPN. However, sIPN product development introduces many unforeseen challenges that are particularly unfamiliar to most academic research institutions. These challenges stem from the divergent goals of an academic lab and an industrial medical device company. The sIPN was originally engineered as an easily modifiable tool to study cell-material interaction during wound healing. This binary material with modified gelatin and PEG demonstrated potential for use in drug delivery and wound treatment in animal wound models. However, when considering manufacturing or commercial usage, many other aspects should also be addressed. For example, manufacturing introduces challenges associated with scale-up, sterility, and endotoxin management. Initial data indicate that the sIPN can be developed for cell-based therapy. However, many key challenges should be addressed through future studies. For example, the use of

allogeneic cells and natural components (*i.e.*, collagen, cell culture medium) during production may impart added risk of transferring potentially infectious or immunogenic agents to experimental animals or human patients. Limited clinical use of current commercially available skin substitutes can

TAKE-HOME MESSAGE

Basic science advances

Skin regeneration is a complex process. PEG + gelatin sIPN provides an ideal platform for fundamental research in wound healing. Independent or combined with emerging approaches such as tissue engineering and stem cell therapy, sIPN is a promising technology to enable advanced wound healing modalities.

Clinical science advances

sIPN has the *in situ* forming ability to support its use in large and irregular wounds with complex contours and crevices. The sIPN platform is also highly functionalizable to address co-morbidities and facilitate the ease of use including clinical adaptation and patient compliance. The extensive preclinical data suggest its potential use in advanced wound care.

Relevance to clinical care

Although there are many choices in primary dressings, a low-cost, easy-to-use, and biofunctionalizable biomaterial that form an intimate contact with the wound bed while maintaining a moist healing environment is still the target of active wound regeneration research that can be met with sIPN.

be attributed to the high manufacturing and product costs, and difficulties with consistent quality control. Hence, a multidisciplinary team approach is crucial to tackle these challenges in the translation of promising medical device technologies.

FUTURE DEVELOPMENT OF INTEREST

The sIPN is currently being manufactured and validated for an initial clinical safety study. A large body of evidence has shown PEG + gelatin based *in situ* forming that sIPN has potential for clinical application as an effective wound treatment. Initial clinical safety testing in the base sIPN, without loaded drug or encapsulated cells, is expected to be completed in 2011. Future development work may

be dedicated to developing the sIPN as a combination product.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This work was supported by a grant (NIH RO1EB6613) from NIH as well as grants from UW-Madison I&EDR, UW-Madison Department of Surgery, Matrilab LLC, and Wisconsin Alumni Research Foundation.

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. No ghostwriters were used to write this article.

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