Fusion factor

By Kai-Jye Lou, Staff Writer

Researchers at **Massachusetts General Hospital** have developed self-assembling nanoparticles consisting of a fusion protein containing an elastin-like peptide and keratinocyte growth factor, two key components of skin remodeling.¹ The nanoparticles could be a therapeutic option in indications like chronic wound healing, and the MGH team plans to evaluate similar elastin-like peptide-based fusion protein combinations for this indication.

Previous studies have suggested that keratinocyte growth factor (KGF) could help accelerate wound healing.² **Swedish Orphan Biovitrum AB** already markets a recombinant human KGF, Kepivance palifermin, to treat severe oral mucositis. The company acquired the biologic from **Amgen Inc.** in 2008. Kepivance is delivered via i.v. infusion.

However, a major drawback to topical treatment with KGF and other growth factors is their limited bioavailability at the injury site, which makes repeated applications necessary and the overall treatment prohibitively expensive.

A team led by Martin Yarmush, director of the Center for Engineering in Medicine and professor of surgery at MGH, set out to improve KGF's bioavailability and efficacy by optimizing its delivery and pairing it up with a complementary skin remodeling factor—elastin-like peptide (ELP).

The idea was based on previous data showing that fusing a therapeutic protein to an ELP—a subunit of tropoelastin—could improve delivery of the protein.^{3,4}

Tropoelastin is the precursor to elastin, a key component of elastic skin fibers. Previous skin remodeling studies have suggested that elastin could be useful in dermal regeneration.^{5,6} In fact, elastin is one of the components of AlloDerm, an acellular human tissue matrix used for third-degree burns, periodontal surgery and plastic and reconstructive surgery. The skin substitute is marketed by the LifeCell Corp. unit of **Kinetic Concepts Inc.**

In addition to exhibiting some of elastin's therapeutic effects, ELPs also have good long-term stability and can rapidly and easily self-assemble into insoluble nanoparticles in response to temperature changes. This property makes ELPs amenable to temperature cycling-based purification methods, which according to Yarmush could have less than half the cost of chromatography- and antibody-based methods.⁷

The team engineered a plasmid vector encoding a fusion protein

of an ELP and KGF and introduced the vector into *Escherichia coli*. The resulting fusion protein retained the key characteristics of ELPs: long-term stability and the capacity for temperature-induced self-assembly into insoluble nanoparticles.

Studies in cell lines showed that the KGF-ELP fusion protein retained the biological activities of both its constituents, such as KGF's ability to promote keratinocyte proliferation and ELP's ability to promote fibroblast proliferation. Both cell populations are important in wound healing.

Next, studies in mice with diabetic chronic wounds showed that a single application of a hydrogel containing the self-assembling KGF-ELP nanoparticles increased wound healing compared with application of hydrogels that contained a non-nanoparticle mixture or either agent alone.

Moreover, skin formed in response to the nanoparticle-containing hydrogel was histologically similar to normal skin. In contrast, skin formed in response to hydrogels containing KGF or ELP alone or as a mixture appeared abnormal.

Results were published in *Proceedings of the National Academy of Sciences*.

"We found that the ELP-KGF nanoparticles produced the most normal-looking skin," said Yarmush, who is corresponding author on the paper. "With ELP alone or free KGF plus ELP, you get a very thick dermis, which is not characteristic of normal skin."

"Proper wound healing requires coordination between the dermal and epidermal repair and regeneration processes—these nanoparticles may indeed work by targeting both skin

components," added François Berthiaume, an associate professor in the Department of Biomedical Engineering at **Rutgers University**.

Wounds and beyond

Yarmush said MGH's nanoparticles could be combined with existing wound care products to improve clinical outcomes. "There are several products on the market for wound care like AlloDerm and the Integra products," he told *SciBX*. "Certainly, you could consider instilling these nanoparticles into these skin substitutes to see if they will produce a better result."

Integra LifeSciences Holdings Corp. markets the Integra series of dermal regeneration and wound dressing products.

Integra and Kinetic Concepts both declined to comment.

Berthiaume agreed that such combinations could be feasible because the nanoparticles are so small that they can be incorporated into the porous matrices of the approved skin substitutes.

"It would also be interesting to study the potential usefulness of the nanoparticles for deep acute wounds that are prone to scarring, which is especially relevant in burns," he said.

Berthiaume said the platform also could be used outside the wound-healing space.

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**—François Berthiaume,
Rutgers University**

“For example, there are studies evaluating the local injection of growth factors, like SDF-1, to enhance heart muscle regeneration,” he told *SciBX*. “The nanoparticles may help keep the growth factor in the vicinity of the injection site and release the active factor over time. Other applications could be incorporated into decellularized matrices to promote cell attachment, proliferation and differentiation of cells seeded in such matrices.”

Nevertheless, Yarmush said his group remains committed to the wound-healing indication, even though it has been very difficult to show improvement over best standard care in clinical trials, and plans to use the platform to create and evaluate other fusion protein combinations.

“You could think about using this platform to create a number of fusion protein combinations with other growth factors,” he told *SciBX*. His group is considering chemokine CXC motif ligand 12 (CXCL12; SDF-1) and platelet derived growth factor (PDGF) as potential candidates for fusion with ELP.

“Since during wound healing several growth factors are involved, a combination therapy comprised of more than one growth factor will be most efficacious,” added Yarmush. “An ideal particle would be a heterogeneous particle comprised of several other important growth factors, like SDF-1 or PDGF, that may act synergistically and

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**—Martin Yarmush,
Massachusetts General Hospital**

improve the prognosis of patients with chronic wounds.”

MGH has filed for a provisional patent covering the nanoparticle generation platform, including the KGF-ELP nanoparticles. The work is available for licensing.

Lou, K.-J. *SciBX* 4(4); doi:10.1038/scibx.2011.94
Published online Jan. 27, 2011

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COMPANIES AND INSTITUTIONS MENTIONED

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