Non-addictive pain therapeutics by sensory neuron targeting

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1. Opioids are prescribed to treat chronic pain in a significant proportion of osteoarthritis patients. Over 25% of the US population over 60 years old has some form of osteoarthritis and the prevalence is increasing faster than any other noncommunicable disease. Opioids are inadequate because a critical level of substance abuse disorders (SUDs) are developed as a result. Opioids simply mask pain, have short duration, and more importantly, cause addiction and loss of human life. A safe, long acting pain drug is needed to prevent SUDs by displacement of opioid use. Neurocarrus seeks to develop a new strategy to treat chronic OA pain that will improve patient outcomes over current clinical practices that emphasize SUD-causing opioids. This project will shift the paradigm to that of safe, localized medical interventions that specifically disrupt sensory neuron pain signals that cause chronic pain.

Through the study of neurobiology and protein engineering, Neurocarrus seeks to combat the opioid epidemic by engineering alternative medications. A long acting protein therapeutic called C2C is being developed. This protein will not cause addiction because it specifically targets peripheral sensory neurons and is administered locally (not systemically). It will not affect motor neurons or the central nervous system. C2C has already shown significant efficacy In a mouse model of inflammatory pain when compared head-to-head with opioids. Initial information has been obtained verifying desirable dosing, duration, and readministration. To meet the currently perceived need of osteoarthritis patients, a topical formulation is required to address the complex nature of neurobiology, pain, and safety profiles. The target of C2C is neuronal actin, a central regulator of inflammatory pain. Actin is directly associated with ion channels necessary for pain signal origination and transmission such as TRPV4 and Nav1.8. Expression of β-actin, the targeted enzymatic substrate of C2C, increases under inflammatory conditions encountered in OA. The purpose of C2C is to disrupt the excess actin formed during inflammation. Actin disruption has been accomplished using small molecule analog drugs in animals to decrease pain behaviors. However, these drugs do not have adequate safety profiles. By development of C2C, a new tool will be available to treat pain and to study actin as a central mechanism in the origination of pain that leads to chronic arthritis conditions.

We propose here to transform the thetreatment of OA pain by combining the C2C protein with transdermal drug delivery technology. This is necessary because C2C is a large molecule, a protein, that cannot otherwise cross the skin. The rationale for this project is that while topical analgesics treat short term mild to moderate OA pain, they are not effective in the treatment of chronic pain. In addition, they have only short duration, are only acceptable for mild to moderate pain, cannot be taken with oral NSAIDS due to gastrointestinal complications, as well as causing burning and stinging at the treatment site, seizure, CNS depression and cardiovascular toxicity. Long-term use of topical analgesic and anesthetic agents is not currently recommended. Neurocarrus currently anticipates the need for safe, long term, topical treatments.

Funds for a minimal viable proof of transdermal C2C delivery in an animal would form the basis of intellectual property for a highly investible startup company. Use of C2C as a tool to disrupt actin in the sensory neuron will also provide a neurobiological tool to study the role of actin in pain signaling and the development of chronic pain. C2C may provide an alternative to opioids which cannot treat the patient population with severe OA pain. C2C may obviate invasive and untargeted techniques that destroy damaged nerves and the surrounding tissues. C2C is a first in kind strategy to accomplish a neuron subtype-specific intervention that may provide relief to complex pain syndromes. Actin remodeling conducted in a neuron subtype targeted manner by C2C treatment may provide a reduction in OA pain without the side effects of tissue destruction or short duration of efficacy.

2. **B. Pavlik (PhD 2017):** Rigorous academic preparation and impactful research productivity forms the basis of my future potential as a biomedical scientist. First, I obtained a BS in Genetics from the University of California, Davis. This experience provided my fundamentals of biology in order to understand medical need. From this foundation, I went through a challenging master's program in the applied life sciences. This integrated program taught scientists and engineers how biotechnology is integrated into society. Next, I entered a Chemical and Biomolecular Engineering PhD program to obtain rigorous technical training. The sum of these experiences has provided a unique platform for me to stand on. My research focuses on protein engineering for the purposes of biomedical applications. I use a combination of molecular modeling and genetic engineering approaches to generate unique cell lines to produce unique proteins for biomedical applications. My additional experience with genetics and biotechnology of extremophiles has contributed to my protein research by providing additional avenues of investigation into protein expression, structure and function. During the course of my PhD thesis research, partially funded by the Defense Threat Reduction Agency, I designed and produced the C2C protein for the purpose of drug delivery to peripheral neurons. This work led to the development of C2C and publication of these findings in Nature Scientific Reports.

P. Blum (PhD 1986): My current research program emphasizes the function of diverse proteins combining in vivo and in vitro approaches. I combine genetic engineering approaches that modify target genes to change expression or composition of proteins, with protein purification and biochemical analysis. My laboratory works on many organisms but the common theme is to integrate data to achieve a mechanistic understanding of protein function. This includes toxin-protein repurposing for pain control treatment, protein post translational modification, various transcription factors, transporters and metabolic enzymes. As PI or co-I on grants funded by NIH, NSF, DOE, DOD and other agencies, my lab has made fundamental contributions about cell biology from archaea to bacteria to eukaryotic systems.

3. The product prototype will be a reformulated version of C2C that is amenable to microneedle transdermal drug delivery. For the consumer, this would consist of a cream that is applied after a commercially available microneedle roller is used to pretreat the area. Further development of the product would lead to an over the counter patch. The C2C cream or patch delivering C2C with microneedle technology would be applied to inflamed joints for treatment of OA pain.

This application seeks to develop a topical analgesic strategy to treat OA pain that will improve over current clinical practices that emphasize opioids and invasive methods. The C2C system specifically targets neurons, and is engineered to disrupt pain signaling mediated by F-actin. This system confers neural specificity to the under-utilized C2 toxin, known to exhibit profound actin remodeling effects without activation of apoptosis in mammalian neurons. C2C was used to deliver fluorescently labeled payloads quantified by fluorescence activated cell sorting (FACS) and found to be dependent on artificial enrichment of cells with the polysialoganglioside receptor, GT1b. Visualization by confocal microscopy showed a dissociation of payloads from the early endosome indicating translocation from early endosomes. C2C was then delivered to human glioblastoma A172 and synchronized HeLa cells. In the presence of the fusion protein, native cytosolic enzymatic activity of the enzyme was observed and found to be GT1b-dependent. In primary cell culture, C2C preferentially effects sensory neurons not motor neurons using an *in vitro* chicken model and exhibited equivalent potency towards mammalian sensory neurons. C2C may enable delivery of other therapeutics to neurons and be of use in neurobiology research. Expanded knowledge of the specificity of binding, endocytosis and neuron-subtype specific effects would allow, for the first time, the development of a much-needed approach to neuron-subtype drug delivery with potential impact on neurobiology research.

Here, it is proposed to conduct *in vivo* transdermal studies with a novel C2C formulation and commercially available microneedles to achieve the minimum viable proof of a non-addictive topical chronic pain treatment for OA pain. *In vitro*, C2C delivers an actin polymerization inhibitor to peripheral sensory neurons, and *in vivo* subcutaneous injections block pain sensation. These promising results offer a new strategy to control pain without the potential for addiction. Transdermal technologies were considered (Table 1) and evaluated. Microneedles were chosen as the best fit to obtain a minimum viable proof. Murine hind limb transdermal applications using microneedles will also provide the most accurate prediction of sensory neuron delivery specificity and pain thresholds in a therapeutic setting because of currently available technology. Transdermal targeting of murine peripheral neurons by C2C will determine preliminary dosing effects with an *in vivo* inflammatory pain model. Transdermal application to the hind limb of a test animal will be conducted using a commercially available microneedle roller to perforate the skin. The reformulated C2C cream will be applied topically and allowed to penetrate the skin. Multiple applications or higher doses may be necessary to achieve a measurable therapeutic effect. After application of C2C, formalin will be injected in the same manner used to evaluate successful subcutaneous C2C pain inhibition. Mouse behaviors associated with formalin induced chemical irritation will then be scored. By comparison of injection and topical transdermal delivery, a basis for therapeutic use will be achieved. In addition, commercial potential will be predicted and provide actual pain threshold model data. Animals will also be evaluated for motor impairment using established digit abduction scoring methods. These pain control and motor impairment data will provide a detailed preliminary picture of C2C *in vivo* activity in regard to both sensory and motor neurons to form minimum viable proof for a topical C2C product. The proposed approach will maximize use of each animal to correlate dosing with histological data and physiological side effects. If established, specific disruption of sensory neurons in vivo using transdermal methods would support further studies with the long-term goal of improving OA pain treatment outcomes.

Table 1. Comparison of technologies for C2C transdermal drug delivery

Transdermal Method	Principle	C2C Compatibility rating	Rating justification
***Microneedles (preferred system)	Direct penetration	+++	Straight forward, provides access to deep skin layers. Can be used in a patch drug delivery system or by pretreatment with microneedle rollers.
Iontophoresis	Local electric current moves charged molecules	++	Needs specialized electrical equipment and that may interrupt neuron signaling
Thermal ablation	Heat makes pores	++	Complicates pain behaviors, requires specialized equipment.
Radiofrequency ablation	RF makes aqueous micropathways	+	Specialized equipment needed
Microdermabrasion	Ablative material such as sodium bicarbonate crystals scrub off dead and dull surface skin cells.	+	Does not provide access to deep skin layers.

Laser-assisted	Laser creates micropores and a patch is applied	++	Specialized equipment needed.
Sonophoresis	Acoustic waves	+	Specialized equipment needed.
Jet systems	High velocity by propellant of formulation	+	Needs more sophisticated propellant system

4. The methods used to determine whether the product is needed by the target audience and whether that audience would be willing to pay for the product are derived from a set of principles shared by Steve Blank and the publication *Biodesign: The process of innovating medical technologies.* First and foremost, the use of primary research will be utilized by study of patient settings and outcomes. By conducting and documenting observations and interviews with patients about their treatment options for OA, the Neurocarrus team will be able to refine the problem of OA pain and gain insight into a larger population by refining need statements. Needs will be confirmed to be solution independent to assure that a new solution can be formulated. The scope and category of each need will be used to develop criteria for solutions.

Needs in OA pain will be screened to determine whether the audience will pay for the intended product. Disease state fundamentals such as anatomy and physiology will be assessed to provide context to understand the normal anatomy and physiology of joints afflicted by OA. Pathophysiology of the disease will be studied to address disease function, causal factors and disease progression. The clinical presentation will be profiled to understand the patient that is experiencing OA pain. Clinical outcomes will provide current morbidity rates associated with OA pain. Epidemiological information will then be gathered to improve the assessment of the economic impact of the needs for OA pain medications. This information will be used to determine the overall cost of OA pain on the medical system. This information will be assessed and summarized into an overview for review with the target audience to confirm that the gathered information is accurate and precise. After this period of observation and analysis of the biomedical needs, an overview of solutions options will be determined by evaluation of current clinical and economic solution profiles. These profiles will be explored to understand why and when each solution is advocated and at what frequency. Emerging solution profiles will also be evaluated to understand how quickly new solutions are already coming to the market. This will result in a landscape of existing solutions. Existing solutions and shortfalls will be considered to provide context and benchmarks to proposed solutions. Stakeholders will be identified and the costs and benefits of these stakeholders will be evaluated to gain an understanding about support or rejection of the intended innovations. Stakeholders will be classified and assessed for the degree at which they are central to the decision-making process to adopt a new innovation. Then the market will be landscaped enable Neurocarrus for competitive positioning as a treatment provider. The dynamics of market segments will be analyzed to identify costs and market power. A price for a new solution will be determined based on the stakeholders, who will be paying, and willingness to pay by evaluation of comparable devices, financial analysis and interviewing stakeholders. Market assessment and needs will inform potential investors that the opportunity exists. These activities are currently being pursued through the engagement of doctors and medical staff for each step of the process from the manufacture of a future product through patient use. These interactions will continue over the next four months. The majority of these interviews will be conducted in the San Francisco bay area through the use of phone, email and personal visits. The hospital systems of Sutter, Alta Bates and Kaiser will be considered for observation and market research. These activities will be conducted by Ben Pavlik and Paul Blum and anticipated new hires.