gene-editing composition, wherein the composition causes expression of IL-1a and IL-1β to be silenced or reduced in a portion of a lame joint's synoviocytes, chondrocytes, synovial macrophages, or synovial fibroblasts.

[0019] In some embodiments, the above method further comprises one or more features recited in any of the methods and compositions described herein.

DESCRIPTION OF THE DRAWINGS

[0020] The presently disclosed embodiments will be further explained with reference to the attached drawings. The drawings shown are not necessarily to scale, with emphasis instead generally being placed upon illustrating the principles of the presently disclosed embodiments.

[0021] FIG. **1**A illustrates an agarose gel electrophoresis analysis of 100 ng mouse DNA (gBlocks, Integrated DNA Technologies) designed against the *Mus musculus* Il1a and Il1b genes, cleaved by 0.5 μg SpyCas9 (TrueCut™ Cas9 protein v2, ThermoFisher Scientific) and 200 ng Phosphorothioate-modified single guide (sg)RNAs targeted against the Il1a gene (#43-46) and Il1b gene (#47-50) in vitro; FIG. **1**B illustrates an agarose gel electrophoresis analysis of 100 ng mouse DNA (gBlocks, Integrated DNA Technologies) designed against the *Mus musculus* Il1a and Il1b genes, cleaved by 0.5 μg SauCas9 (GeneSnipper™ Cas9, BioVision) and 200 ng Phosphorothioate-modified guide sgRNAs against the Il1a (#51-53) and IL1b (#54-56) genes.

[0022] FIGS. **2**A-**2**D illustrate graphs displaying editing efficiencies of SpyCas9 and SauCas9 used with a range of guide RNA's in J774.2 ("J") and NIH3T3 ("N") cells; FIG. **2**A: in vivo cleavage of Il1a, edited with 4×sgRNAs (Spy Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); FIG. **2**B: in vivo cleavage of Il1b, edited with 4×sgRNAs (Spy Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); FIG. **2**C: in vivo cleavage of Il1a, edited with 3×sgRNAs (Sau Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); FIG. **2**D: in vivo cleavage of Il1b, edited with 3×sgRNAs (saCas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); editing efficiencies determined using deconvolution of Sanger sequencing traces (ICE tool, Synthego) of each pool.

[0023] FIG. **3** illustrates GFP expression measured using the IVIS system. Flux values were based on a region of interest centred on the animal's injected knee joint. Data are presented as mean (SD) for four specimens per group.

[0024] While the above-identified drawing sets forth presently disclosed embodiments, other embodiments are also contemplated, as noted in the discussion. This disclosure presents illustrative embodiments by way of representation and not limitation. Numerous other modifications and embodiments can be devised by those skilled in the art which fall within the scope and spirit of the principles of the presently disclosed embodiments.

DETAILED DESCRIPTION OF THE INVENTION

[0025] As described herein, embodiments of the present invention provide compositions and methods for improving joint function and treating joint disease. In particular embodiments, compositions and methods are provided to gene-edit synovial fibroblasts, synoviocytes, chondrocytes, or synovial macrophages to reduce expression of inflammatory cytokines, for example, IL-1α, IL-1β, TNF-α, IL-6, IL-8, IL-18, one or more matrix metalloproteinases (MMPs), or one or more component of the NLRP3 inflammasome. Embodiments are used for treating osteoarthritis and other inflammatory joint diseases. Embodiments are further useful for treating canine lameness due to osteoarthritis. Embodiments are further useful for treating equine lameness due to joint disease. Embodiments are also useful for treating post-traumatic arthritis, gout, pseudogout, and other inflammation-mediated or immune-mediated joint diseases.

Definitions

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference in their entireties.

[0027] The term "in vivo" refers to an event that takes place in a subject's body.

[0028] The term "in vitro" refers to an event that takes places outside of a subject's body. In vitro assays encompass cell-based assays in which cells alive or dead are employed and may also encompass a cell-free assay in which no intact cells are employed.

[0029] The term "ex vivo" refers to an event which involves treating or performing a procedure on a cell, tissue and/or organ which has been removed from a subject's body. Aptly, the cell, tissue and/or organ may be returned to the subject's body in a method of surgery or treatment.

[0030] The term "IL-1" (also referred to herein as "IL1") refers to the pro-inflammatory cytokine known as interleukin-1, and includes all forms of IL-1, including IL1-α and IL-1β, human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-1α and IL-1β bind to the same receptor molecule, which is called type I IL-1 receptor (IL-1RI). There is a third ligand of this receptor: Interleukin 1 receptor antagonist (IL-1Ra), which does not activate downstream signaling; therefore, acting as an inhibitor of IL-1α and IL-1β signaling by competing with them for binding sites of the receptor. See, e.g., Dinarello, *Blood* 117: 3720-32 (2011) and Weber et al., *Science Signaling* 3(105): cm1, doi:10.1126/scisignal.3105cm1. IL-1 is described, e.g., in Dinarello, *Cytokine Growth Factor Rev.* 8:253-65 (1997), the disclosures of which are incorporated by reference herein. For example, the term IL-1 encompasses human, recombinant forms of IL-1.