**Introduction:** Pathologies of the tendon are relatively commonplace, yet current treatments for many of these problems are often suboptimal. Though most tendons have the ability to heal spontaneously after injury, the scar tissue that is formed is almost always mechanically inferior and therefore much less able to perform the functions of normal tendon, and is also more susceptible to further damage. Because the formulation of effective treatments for tendon injuries based on traditional tissue-level reparative procedures, surgical or otherwise, has presented such a problem to clinicians in the field, much research has been directed toward understanding the mechanisms of tendon healing at the molecular level. This has ultimately been in an effort to develop therapies to facilitate tendon healing through the use of individual molecules or groups of molecules known to have beneficial roles in the process.

The healing cascade within the tendon is initiated, sustained, and eventually terminated by a large number and variety of molecules. Recent studies, however, have shown that the free radical nitric oxide (NO) has been shown to be a particularly important regulator of gene expression during many of these processes. Elsewhere NO has key roles in processes as varied as neuronal signalling and as an effector molecule for the immune system. It is produced by a class of enzymes known as the nitric oxide synthases (NOS), whose three isoforms all catalyse the conversion of L-arginine to NO and citrulline. NO has been found to be significantly upregulated in animal tendon damage models from the first day post-injury, and is thought to remain in abundance throughout the healing process. Though its important regulatory roles in tendon healing have been clearly shown, exactly which genes it exerts its effects on remains mostly unknown.

The current study used microarray analyses to investigate the expression of 4,224 genes in cultured tendon fibroblast cells after stimulation with NO delivery. The added nitric oxide was measured by: (i) Establishment of Fibroblast Cell Culture: Human rotator cuff tendon tissue was harvested from consenting patients during routine reparative surgery. The tissue was incubated in a collagenase solution at 37°C for approximately 12 hours to degrade extracellular matrix materials, and the fibroblasts released were used to establish cell cultures. All experiments utilised cells at their third passage, and control cells were used for microarray expression analysis. Data analysis showed that several hundred genes had expression significantly up- or down-regulated compared to controls. Among these are several genes which may have important roles in tendon healing, including transcriptional and translational activators of growth factors, cellular migration signals, regulators of angiogenesis, morphogenic proteins, and extracellular matrix proteins.

**Methods:** (i) Establishment of Fibroblast Cell Culture: Human rotator cuff tendon tissue was harvested from consenting patients during routine reparative surgery. The tissue was incubated in a collagenase solution at 37°C for approximately 12 hours to degrade extracellular matrix materials, and the fibroblasts released were used to establish cell cultures. All experiments utilised cells at their third passage, and control cells were used for microarray expression analysis. Data analysis showed that several hundred genes had expression significantly up- or down-regulated compared to controls. Among these are several genes which may have important roles in tendon healing, including transcriptional and translational activators of growth factors, cellular migration signals, regulators of angiogenesis, morphogenic proteins, and extracellular matrix proteins.

(ii) SNAP Stimulation of Cultured Fibroblasts: Two different methods were used to deliver nitric oxide to the cultured fibroblastic cells. The first used the chemical NO donor S-nitroso-N-acetyl-penicillamine (SNAP) to culture media, and transfection with the inducible nitric oxide synthase (iNOS) gene via an adenoviral vector. Following stimulation of fibroblast cells, RNA was extracted from each experimental and control group, and used for microarray expression analysis. Data analysis showed that several hundred genes had expression significantly up- or down-regulated in each NO-stimulation experiment compared to the controls. Further filtering produced a short list of 21 genes whose expression was at least two fold different from controls and showed consistent results over all 8 targets in both NO-stimulation experiments. Upregulated genes include transcription factors (such as Class II POU Domain-containing transcription factors, Inhibition Factor EIF1A, and TFI1), extracellular protein and structural molecules (such as Clatherin and Myosin), transport proteins (such as Synaptophysin 1a), cell division regulators (such as CDC10), signal-transducer proteins (such as Guanine Nucleotide Binding Protein and beta-Dystrobrevin) and morphogenic protein receptors (such as Type II BMP receptor). (Complete list not shown).

**Discussion:** Microarray technology is a powerful tool allowing the simultaneous comparison of thousands of genes of two cell populations at a single point in time. This study used microarray analyses to examine the effects of nitric oxide, a ubiquitous signal molecule shown to be important during tendon healing, on cultured fibroblast cells. Two different methods of NO delivery were used to stimulate fibroblast cells: transfection with adenovirus containing the iNOS gene, and addition of the chemical NO donor SNAP. The changes in gene expression that resulted from each NO stimulation were measured and compared. Of the 21 genes whose expression were most markedly affected, many could be predicted to play important roles during tendon healing, particularly transcriptional and translational initiation factors, signalling proteins, and structural molecules, though few of these have been directly linked to the action nitric oxide. Though it is difficult to distinguish which of these genes are directly stimulated into upregulation by NO and which are downstream effects, these results are still extremely useful in explaining exactly why nitric oxide is such an important signal during tendon healing.