

Osteoarthritis prevention using BCL2



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Osteoarthritis (OA) is a chronic, progressive illness that affects millions worldwide, primarily those aged 45 and above. Commonly known as “wear and tear” arthritis, it is caused by the degradation of cartilage in the joints. There is no cure for the disease, but there are methods to manage pain and symptoms. There are many challenges to curing osteoarthritis, especially in the development of Disease Modifying Osteoarthritis Drugs (or DMOADs). Most of these issues come from patient testing and the limitations of conventional technology used in trials. Here, we propose a solution for treating osteoarthritis by using a gene called BCL2. BCL2 is responsible for blocking the apoptosis of specific cells. We believe that we could utilize BCL2 to prevent the death of chondrocytes—cells responsible for producing and maintaining cartilage—and inhibit caspase activity, including Caspase-1. Caspase-1 is responsible for the maturation of IL-1 β , which is widely known for degenerating cartilage. Therefore, the overproduction of IL-1 β stimulates osteoarthritis. *Saccharomyces cerevisiae* will be transformed with the BCL2 gene, and we will then test it for purity using PCR. Afterwards, we will test its response to IL-1 β . Finally, our solution will be made into an injection that can be applied to the joint. If our solution works as intended, then it would slow down the progression of osteoarthritis and hopefully restore the patient’s quality of life.

Keywords: Osteoarthritis, BCL2, BAX, Interleukin-1, IL-1 β , Caspase-1, cartilage degradation

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Background

Osteoarthritis is the degeneration of articular cartilage that covers the joints. (Figure 1) It is a chronic disease that affects over 240 million people worldwide (Centers for Disease Control and Prevention 2020). Treatments can help symptoms and manage the pain, but there is no cure. Factors that can increase one’s risk of getting osteoarthritis include old age, joint injuries, stress, and bone deformities. One of the most common existing solutions is the corticosteroid knee injection.

This type of solution works very quickly to relieve pain caused by an inflamed joint (Metcalfe 2009). Although corticosteroid knee injections are fast-acting, their effect is only temporary, and pain will come back after a few weeks. Non-medical methods of treating osteoarthritis to reduce pain include physical and occupational therapy, exercise, and assistive devices (Mayo Foundation for Medical Education and Research (MFMER) 2020). These techniques are frequently doctor-recommended and are helpful in temporarily easing symptoms.

Other solutions that are being developed are Disease Modifying Osteoarthritis Drugs (DMOADs). The primary goal of DMOADs is to slow the progression of OA by inhibiting the structural damage of the disease and improving function. Right now, there are no licensed DMOADs as the development process is long and challenging. For instance, sometimes patients drop out during long-duration trials (Barr and Conaghan 2013). DMOADs will be aimed to treat the aging population and will likely be prescribed alongside other medication. As a result, DMOADs need to show a good safety profile to patients and other drugs. For this reason, DMOADs need to undergo long experiments. Another challenge in DMOAD development is the limitation of conventional radiography. Currently, conventional radiography does not detect early OA changes in the subchondral bone or cartilage, which may delay further trials (Barr and Conaghan 2013). In addition, the course of osteoarthritis is highly variable, therefore it is difficult to produce a preclinical model that reflects all components of human OA (Barr and Conaghan 2013).

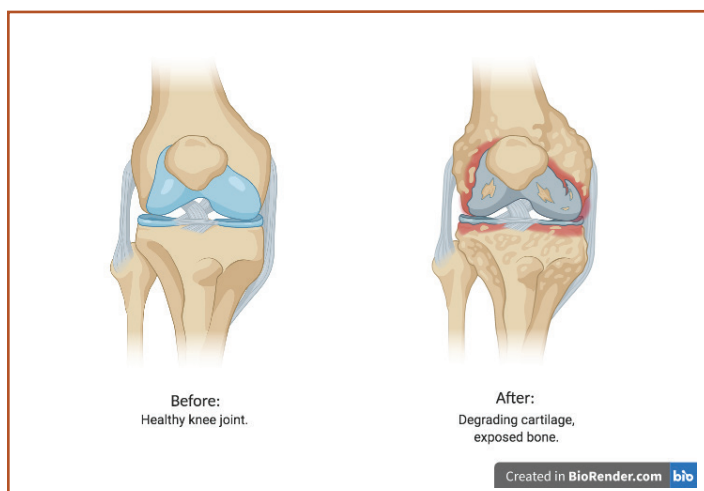


Figure 1. Effects on a knee joint before and after osteoarthritis diagnosis.

One of the key factors that induces osteoarthritis is Interleukin-1 β (IL-1 β). Interleukin-1 is a cytokine that synthesizes matrix metalloproteinases or MMPs (Ling and Bathon 2016), which are responsible for degrading the cartilage. MMPs work at a faster rate than chondrocytes, or active cells that make cartilage. As a result, more cartilage is worn away than the amount being synthesized. Caspase-1, or Interleukin-1 Converting Enzyme (ICE), is a cysteine protease responsible for cleaving pro-IL-1 β into its active form (Giegel and Kostlan 1998). It releases IL-1 β , and produces an inflammatory response. Other osteoarthritis related inflammation can also occur due to external factors that put a lot of pressure on the joint. When the joint is put under physical stress, chondrocytes react by releasing

cytokines that promote swelling (Goldring and Otero 2011).

Two genes involved with osteoarthritis are BCL2 and BAX, also known as BCL2-associated X protein. These two genes are part of the same family and can form heterodimers or homodimers and act as anti-apoptotic or pro-apoptotic regulators in various cell activities (BAX gene - GeneCards 2019). The BCL2 gene is anti-apoptotic, meaning it encodes a mitochondrial membrane protein that blocks the apoptotic processes of some cells (National Library of Medicine (NLM) 2016). It is also responsible for inhibiting caspase activity by preventing the release of cytochrome c from the mitochondria (Figure 2A) by binding to APAF-1 (Figure 2B), the apoptosis-activating factor. BCL2 can reduce inflammation by impairing the activation of the Caspase-1 precursor (CASP1) and the release of IL-1 β (National Library of Medicine (NLM) 2016). On the other hand, the BAX gene is proapoptotic and mediates cell death. It can lead to the release of cytochrome c, which can trigger the activation of caspases.

In osteoarthritis, the apoptosis of chondrocytes is a key factor. Chondrocytes are the only cells in cartilage, and their function is to produce and maintain the cartilage matrix. If chondrocytes commit cell death at an increased rate, the cartilage will wear away, leading to osteoarthritis. Our design will try to inhibit the apoptosis of chondrocytes by using the BCL2 gene. Studies were done to compare the mRNA expression levels of the genes BCL2 and BAX in human OA and normal articular cartilage. In the analyses, it was found that in osteoarthritis patients, there was a significant increase in levels of BAX, which were upregulated by signals that induce apoptosis (Kourtis, Adamopoulos, Papalois, et al. 2018). In addition, the ratio of BCL2 to BAX was much lower in OA than the ratio in normal cartilage (Karaliotas, Mavridis, Scorilas, et al. 2015). This means that in osteoarthritis, there is an excess amount of BAX proteins compared to BCL2 proteins. Based on these studies, we hypothesized that if there were more BCL2 proteins, perhaps more chondrocytes would be preserved and that some of the Caspase-1 activity would be prevented. This would reduce the release of IL-1 β and limit the progression of osteoarthritis. We decided to encode the BCL2 gene into our prototype.

There is research that shows that damage to the BCL2 gene can increase the risk of cancer, including melanoma and follicular lymphoma. This is because the overexpression of this gene and the underexpression of pro-apoptotic genes can lead to an absence of cell death, or the main feature of cancer. We will have to extensively test the effects of the BCL2 proteins on cartilage and other tissues to gain more information. It will also be

imperative that the patient receives the correct dosage so they would not succumb to any detrimental effects

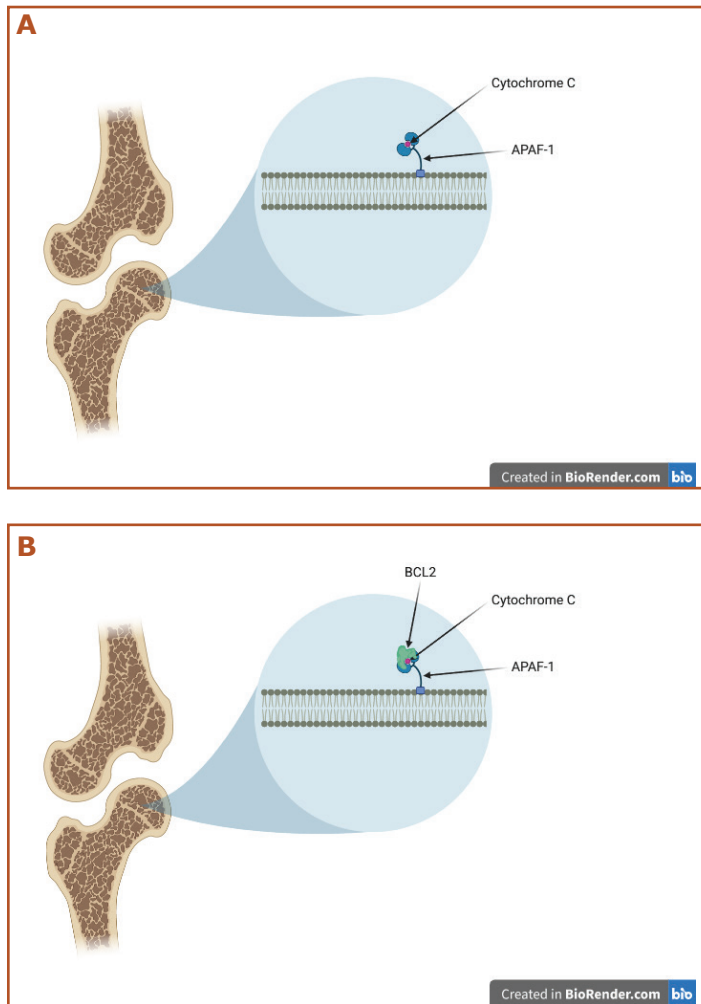


Figure 2. (A) APAF-1 releases Cytochrome C, a protein on the mitochondrial membrane. **(B)** BCL2 blocks the freeing of Cytochrome C, which inhibits caspase activity.

In the future, we would strive to evolve the project to be able to alleviate symptoms, like pain, stiffness, and loss of flexibility, in patients. Right now without testing, we cannot guarantee that this will be a result of treatment.

Systems level

Ideally, an injection of our treatment in the affected area of the body would limit the degradation of cartilage. The apoptosis of chondrocytes plays an important role in osteoarthritis and upregulates BAX proteins. When there is an excess of the proinflammatory cytokine, Interleukin-1 β , MMPs –which degrade cartilage– are produced. Our proposed solution contains the gene, BCL2, which produces proteins that will help balance out the ratio with BAX proteins and will also inhibit the activation of Caspase-1– an enzyme known for producing

IL-1 β . The increase in BCL2 proteins and a decrease in IL-1 β would reduce the deterioration of cartilage.

Device level

We considered many potential hosts for our design, and we eventually chose *Saccharomyces cerevisiae*, or baker's yeast. Since we are using a eukaryotic gene, our chassis also needs to be eukaryotic. *S. cerevisiae* is one of the most widely researched organisms used in synthetic biology and is a common host for genetic material. It can be easily cultured and can be transformed by the addition of genes, which is what we plan to do. The main functional component of our design is that it utilizes the BCL2 gene to create proteins that can prevent chondrocyte apoptosis and the release of IL-1 β after inhibiting Caspase-1. The blue chromoprotein, amilCP, will serve as our reporter and confirm that the proteins are being produced upon the translation of the gene.

Our plan involves growing two cultures of *S. cerevisiae* cells. We will use one culture as our control, and this group should be unable to reproduce. The second culture will be injected with the BCL2 and chromoprotein genes. As the genes are translating, the amilCP gene (the reporter) should be secreting dark blue proteins, indicating that our design is working. Afterwards, the cells in the second culture will be tested for BCL2 by measuring its mRNA expression levels using real-time polymerase chain reaction (PCR) and standardized reverse transcriptase-PeCR (StaRT-PCR). PCR will be used throughout the experiment to reproduce the genes needed for our design. Finally, we will use centrifugation to separate the BCL2 proteins from the yeast cells, removing them from the chassis. The proteins will then be prepared for an injection that can be applied in the joint.

Parts level

Our design features five major genetic components: the pAdh promoter (Sommovilla 2007), a strong ribosome binding site for yeast (Ajo-Franklin 2006b), the BCL2 gene, the amilCP reporter (Sun 2011), and the ADH1 terminator from *S. cerevisiae* (Ajo-Franklin 2006a) from the Registry of Standard Biological Parts (Figure 3).

The pAdh promoter is a strong, constitutive promoter for yeast. It is a DNA sequence that is responsible for the transcription of a gene and depends on the availability of RNA polymerase holoenzyme. We chose to have a constitutive promoter because it is important

that the genes in our design are secreting as many BCL2 proteins as possible.

A ribosome binding site (RBS) is an RNA sequence that ribosomes can attach to and initiate translation. In order for our design to translate, our system will require an RBS sequence and a start codon. An RBS part is needed to create a system that will produce a protein. Yeast ribosome binding sites are known as Kozak sequences and are necessary for beginning the translation of our design. We picked the BBa_J63003 (Ajo-Franklin 2006b) part because it is commonly used for *S. cerevisiae*, has a start codon, and will help maximize our system's products.

The coding sequence will consist of two parts: the BCL2 gene and the gene to encode the blue chromoprotein, amilCP. The BCL2 gene will begin to produce BCL2 proteins, the desired product, during the translation process. The blue chromoprotein reporter is the second part of the coding sequence. It naturally exhibits a strong blue or violet color which is easily visible without any instruments. As the *S. cerevisiae* translates the BCL2 gene alongside the reporter gene, the yeast cells should start to turn color as BCL2 proteins are being secreted. The amilCP should indicate that the system is working, and can help distinguish between functioning and nonfunctioning colonies.

The ADH1 terminator occurs naturally in *S. cerevisiae*. It is a forward terminator, meaning that it will cause transcription to stop when placed downstream of a genetic part. We selected this component because it works well with our design and chassis.

Safety

To safely produce and test our drug, specific precautions must be taken. Close attention will be paid to the quantities of each of our components as well as the work environment. Most laboratory strains of our chassis, *S. cerevisiae*, do not produce toxins that are harmful to humans. They are notably safer than wild-types or commercial strains. *S. cerevisiae* overall is remarkably safer than other eukaryotic chassis as well.

Strains of *S. cerevisiae* known for being fatal are not quite that dangerous compared to other bacteria; at most, if ingested, they may cause mild skin irritation. However, such types will not be used in our design, and to stress lab safety, anti-contamination methods will be used to prevent exposure from potential fumes, gases, and or dusts. It is necessary that we treat any and all live bacteria as a biohazard to ensure protection. Any individuals working to produce our design must wear appropriate lab attire, some of which include safety goggles, gloves, and clothing which does not expose skin. With this, lab hazards, such as spilled chemicals or biological materials, will be minimized. When it comes to centrifugation, it is crucial that all applicable tubes, bottles, and rotors are used. This will ensure that the purification of our design will be faultless.

Another issue that would generate concern is the BCL2 gene itself. Mutations of the gene have been known to be linked to cancer, especially lymphoma. In order to verify that our design will not have any harmful side effects, comprehensive testing will need to be done on cartilage and various tissues. Side effects of our treatment could include rapid tissue growth resulting from a destructive duplication of cells. Once we attain positive results, we hope to test the design in live mammals such as mice. For now, we do not have a full understanding of all the effects of our treatment until we do extended testing.

Discussions

Our project will limit the degradation of cartilage and ultimately help the 240 million people who suffer from osteoarthritis. Some improvements that could help our project is a development in technology that would allow us to see the scope of the degraded cartilage. As of right now, we can only accomplish this through mouse models or clinical trials; however, an improvement in technology would allow for more accurate results.

One challenge we could potentially face is that we cannot guarantee that slowing the deterioration of cartilage will reduce a patient's pain. It cannot be ensured that limiting the degradation of cartilage will completely

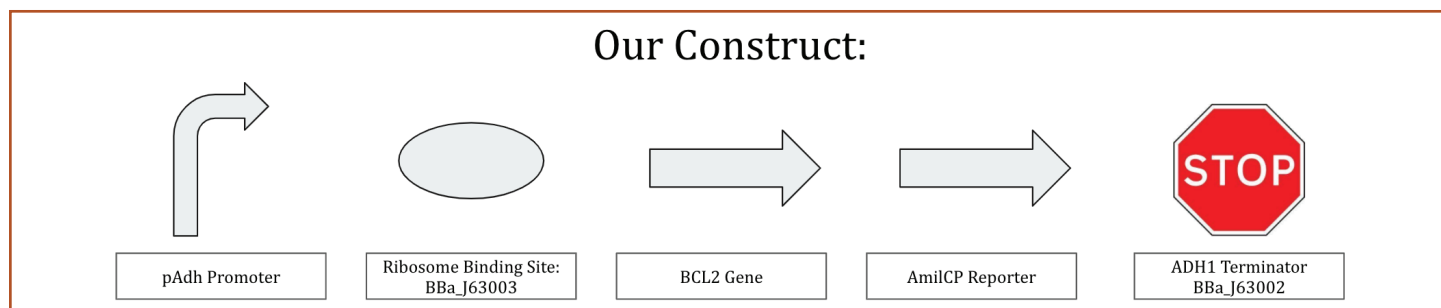


Figure 3. This construct is devised of 5 genetic parts, further explained in the Parts Level section.

restore their quality of life, either. Another significant factor that needs to be considered is how the BCL2 gene in our design will affect other tissues in the body. Mutations in the BCL2 gene are linked to cancer, so intensive testing is needed to study if the gene is being translated correctly without any issues. In addition, we must further test and research the dosage of BCL2, as too much of it can cause lymphoma. We would hope to test these issues using a future mouse model and by experimenting with other tissues. We also hope to advance to a clinical trial.

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References

- Ajo-Franklin C. Part:BBa J63002 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2006a Oct 11 [cited 2020 May 28]. Available from: https://parts.igem.org/Part:BBa_J63002
- Ajo-Franklin C. Part:BBa J63003 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2006b Oct 11 [cited 2020 May 28]. Available from: http://parts.igem.org/Part:BBa_J63003
- Akkiraju H, Nohe A. Role of Chondrocytes in Cartilage Formation, Progression of Osteoarthritis and Cartilage Regeneration. *Journal of Developmental Biology* [Internet]. 2015 Dec 18 [cited 2020 May 28];3(4):177–92. Available from: <https://pubmed.ncbi.nlm.nih.gov/27347486/>
- BioRender [Internet]. Home. BioRender; 2020 [cited 2020 May 28]. Available from: <https://biorender.com/>
- Barr AJ and Conaghan PG. Disease-modifying osteoarthritis drugs (DMOADs): what are they and what can we expect from them? *Medicographia* [Internet]. Medicographia. 2013 [cited 2020 May 28];35:189-96. Available from: <https://www.medicographia.com/2013/10/disease-modifying-osteoarthritis-drugs-dmoads-what-are-they-and-what-can-we-expect-from-them/>
- BAX Gene - GeneCards: | BAX Protein | BAX Antibody [Internet]. Israel: Weizman Institute of Science, Genecards.org;. 2019 [cited 2020 May 28]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=BAX>
- Centers for Disease Control and Prevention. Osteoarthritis (OA) [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2020 [cited 2020 May 28]. Available from: <https://www.cdc.gov/arthritis/basics/osteoarthritis.htm>
- Giegel DA, Kostlan CR. Blocking Interleukin-1 Action. *Annual Reports in Medicinal Chemistry* [Internet]. 1998 [cited 2020 Aug 14];183–92. Available from: <https://www.sciencedirect.com/science/article/pii/S0065774308610830>
- Goldring MB and Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol* [Internet]. 2011 Sep [cited 2020 May 28];23(5):471–8. Available from: <https://pubmed.ncbi.nlm.nih.gov/21788902/>
- Karaliotas GI, Mavridis K, Scorilas A and Babis GC. Quantitative analysis of the mRNA expression levels of BCL2 and BAX genes in human osteoarthritis and normal articular cartilage: an investigation into their differential expression. *Molecular Medicine Reports* [Internet]. 2015 Jun 16 [cited 2020 May 28];12(3):4514–21. Available from: <https://www.spandidos-publications.com/10.3892/mmr.2015.3939>
- Kourtis A, Adamopoulos PG, Papalois A, Iliopoulos DC, Babis GC and Scorilas A Quantitative analysis and study of the mRNA expression levels of apoptotic

- genes BCL2, BAX and BCL2L12 in the articular cartilage of an animal model of osteoarthritis. *Annals of Translational Medicine* [Internet]. 2018 Jun [cited 2020 May 28];6(12):243. Available from: <http://atm.amegroups.com/article/view/19949/html>
- Ling S, Bathon J. Osteoarthritis : Pathophysiology [Internet]. Johns Hopkins Arthritis Center. 2016 [cited 2020 May 5]. Available from: <https://www.hopkinsarthritis.org/arthritis-info/osteoarthritis/oa-pathophysiology/>
- Mayo Foundation for Medical Education and Research (MFMER). Osteoarthritis - symptoms and causes [Internet]. Mayo Foundation for Medical Education and Research (MFMER); 2020 [cited 2020 May 28]. Available from: <https://www.mayoclinic.org/diseases-conditions/osteoarthritis/symptoms-causes/syc-20351925>
- Metcalf E. Injectable medications for knee osteoarthritis [Internet]. WebMD LLC; 2009 [cited 2020 May 28]. Available from: <https://www.webmd.com/osteoarthritis/features/injections-for-osteoarthritis-pain>
- National Library of Medicine. BCL2 gene [Internet]. Bethesda (MD): National Library of Medicine, Genetics Home Reference; 2016 [cited 2020 May 28]. Available from: <https://ghr.nlm.nih.gov/gene/BCL2>
- Sommovilla N. Part:BBa_I766556 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2007 Aug 16 [cited 2020 May 28]. Available from: http://parts.igem.org/Part:BBa_I766556
- Sun L. Part:BBa_K592009 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2011 Sep 18 [cited 2020 May 28]. Available from: http://parts.igem.org/Part:BBa_K592009