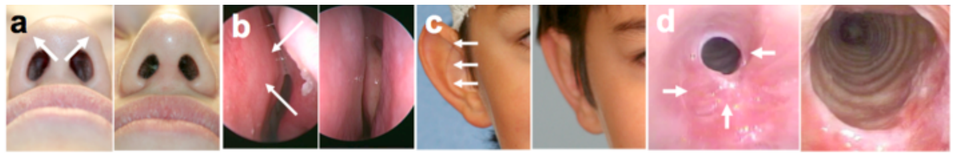
Imaging Proton Diffusion During the Electromechanical Reshaping (EMR) of Cartilage

Introduction: Fundamentals of EMR

Cartilage malformations are attributed to several serious health effects, including respiratory, hearing, and developmental issues. Originating from sources such as congenital defects, accidents, diseases, cancer treatments, and other external factors, these malformations often need to be addressed surgically. The typical procedure to fix the malformation involves "cut-and-sew" surgical techniques, involving the alteration of the macroscopic cartilage structure via carving, scoring, morselizing, and suturing. While these techniques have been refined with the evolution of medicine, the fundamentals of the technique have remained the same for centuries. The technique is invasive and runs the risk of complications with long recovery times.

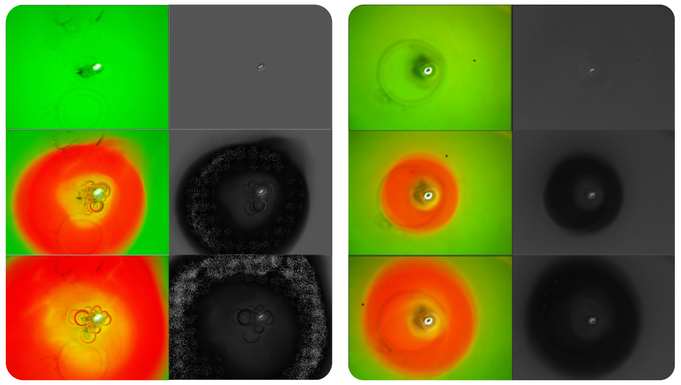


Cartilage malformation of the face: a)severe boxy nasal tip deformity (pre-op left, post-op right); b) deviated septum (left), and after septoplasty (right); c) severely protuberant ear (arrow indicate lack of normal fold) and after surgery (otoplasty, right); d) stenotic tracheal airway (before surgery left, after-right). Arrows point towards regions of deviation or deformity.

EMR—being developed by the Hill Lab, as well as the Wong Research Group at UCI—has the potential to replace these surgeries by transiently altering the cartilage framework. When an electrical current is passed through the electrodes and into the extracellular matrix, water is electrolyzed at the surface of the anode producing protons that then diffuse across the matrix. The protonation of the immobilized anions of proteoglycans alters the ionic bonding network described above and disrupts the structural integrity of cartilage, causing it to lose its rigidity and thereby be reshaped. To achieve the desired shape, the cartilage is aligned with a mold (which can be created using a 3D printer), with electrodes placed along the areas where a new crease or form is desired. After the treatment is over, the tissue can be re-equilibrated to physiological pH using PBS buffer to deprotonate the tissue and restore the ionic bonding matrix, returning the cartilage to its rigid structure.

Early iterations of the technique would often result in damage to the chondrocytes surrounding the inserted electrode. Further studies attributed this damage to reactive oxygen species and hypochlorite generated from oxidative processes within the extracellular matrix, as well as over-acidification near the electrode. A promising solution to these setbacks comes in the form of a looped application of potential to the electrode; using a potentiostat, a galvanostatic-potentiostatic loop is applied. This allows for protons to more evenly diffuse throughout the targeted area of cartilage. Essential to the dosimetry of the treatment hence requires visualization of how protons diffuse throughout an electrolyte solution.

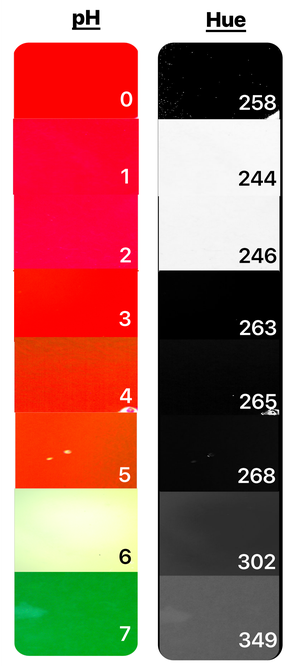
Step I: Extracting Hue Values



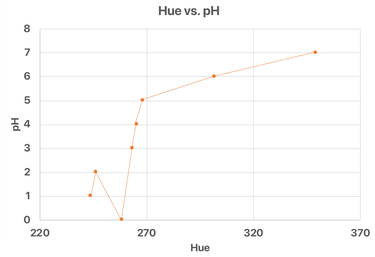
Stills from the original video-captured change in pH (left-side of each panel) and its corresponding hue-representing still (right side in each panel) in two different trials, at 0 seconds (top), 60 seconds (middle), and 120 seconds (bottom). On the left, a constant potential was passed at 2.3V. On the right, the looped application was used, with iterations at 0 and 1.5V.

To observe how protons diffused throughout an electrolyte solution, three drops of universal indicator was placed in a 0.1M electrolyte solution. Universal indicator changes color depending on the pH; this change of color was monitored using an AmScope microscope with an attached camera. A video was taken of the process (colored images), then converted to represent hue values (grayscale images) in order to quantitatively measure the color of solution at various distances and time.

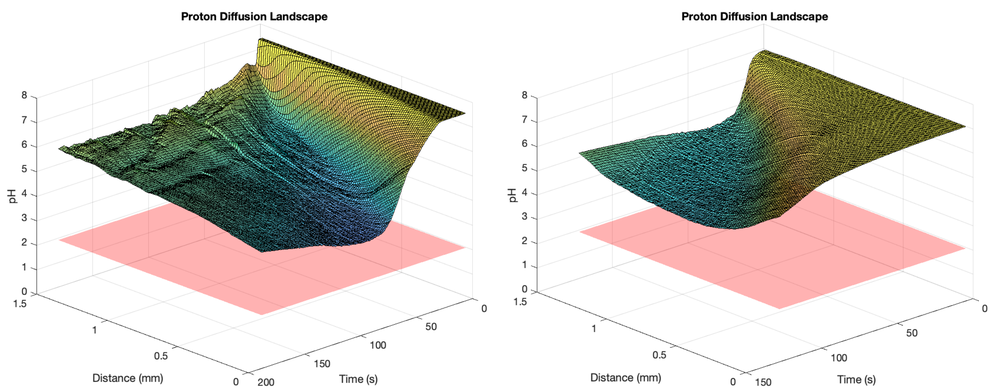
Step II: Converting Hue to pH



Buffer solutions of varying, known acidities were made in order to establish a calibration curve between hue and pH. An image was captured of the solution with the same camera in the same environment. These images were imported into the program FIJI to extract the average hue value from the image. These values, on a scale from 0 to 255, were then plotted against the known pHs in Excel. A piecewise function was plotted as means to convert the hues from the video to pH. As the nature of hue is cyclical, 255 was added to some values to preserve the integrity of am identity function.



Step III: Visualizing Proton Diffusion Over Space and Time



Left: Proton diffusion map during the application of a constant potential passed at 2.3V.
Right: Proton diffusion map during the application of a looped galvanostatic-potentiostatic program with iterations at 0 and 1.5V.

Using FIJI, approximately 100 concentric ring regions of interest were drawn around the electrode, up to a radius of about 1.5mm. Then for each second, the average hue in each ring was calculated by the program, resulting in over 12,000 data points for each video. This data was then imported into MATLAB and plotted on a surface to give a three-dimensional representation of proton diffusion over time. As evident, the looped sequence gives a much more precise diffusion pattern as opposed to the simple-applied potential. This will allow for greater control during the application of the treatment in vivo.

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Funding provided by Occidental College Undergraduate Research Center