Electrospun Collagen Scaffold for Tympanic Membrane Perforations

Ying Li[1], Prof. Wankei Wan[1, 2]
[1]Biomedical Engineering Graduate Program
[2]Department of Chemical and Biochemical Engineering
Western University, London, Ontario, Canada

Tympanic membrane (TM) perforations or ruptured eardrum due to diseases and accidents are common and often require surgical treatment[1]. Current treatment using grafts suffer various limitations and none of these grafts has the microanatomy of the native TM to produce sound quality in the native eardrum. A normal TM includes two layers of orthogonally organized collagen nanofibers which play an essential role in sound transmission[2]. Thus, it is desirable to fabricate collagen-based scaffolds that can replicate the complex microanatomy to reproduce sound quality in the native TM. Among scaffold fabrication techniques, electrospinning allows fabrication of fibers with sub-micron diameter and possible alignments and assemblies of the fibers can mimic the local structure of native TM.

The purpose of this study is to use the electrospinning technique to develop a collagen scaffold that mimics the natural tissue's microanatomy to guide new matrix deposition.
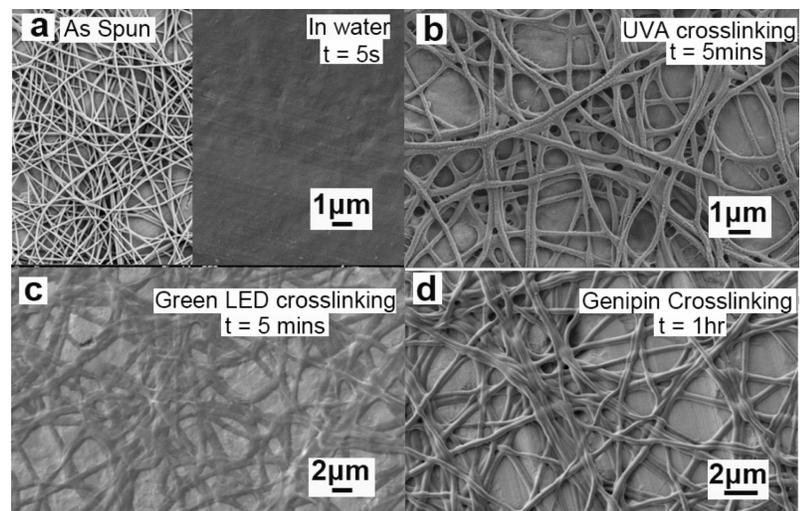
Figure 1: SEM images of (a) as spun collagen fibers/as spun collagen fibers in water for 5 seconds; (b) UV crosslinked collagen fibers in water for five minutes; (c) Green LED crosslinked collagen fibers in water for five minutes; (d) genipin crosslinked collagen fibers in water for one hour

Electrospinning was carried out in an environment controlled chamber. Experimental parameters such as needle to collector distance, supplied voltage, and solution flow rate were adjusted to obtain fibers with desired morphology and sizes.

Fibers were crosslinked using various methods [3]. The aqueous stability of the fibers after crosslinking was tested by placing the samples in distilled water for various amount of time. Two layers of perpendicularly aligned fibers were collected using rotating mandrel to mimic the fiber orientation in TM.

By placing the as-spun fibers in water for 5 seconds, fibrous scaffold immediately gelled and lost fiber morphology (Fig. 1a). Whereas UV crosslinking, green LED crosslinking and genipin crosslinking enhanced the aqueous stability of the fibers (Fig. 1b-d).

Two-dimensional fast Fourier transform (2D FFT) was used to compare the degree of fiber alignment to the rat TM (Fig. 2a and 2b). Two distinct peaks offset by 90 degree indicate the orthogonal relationship between the two layers and these peaks match the fiber organization of the TM (Fig. 2c).

UV and green LED crosslinking, which include no chemical crosslinking reagents, demonstrate improved aqueous stability of fibers as genipin crosslinking. Two layered aligned fibers shows a close mimicry to the fiber organization in the native TM. With similar structure to the original TM, this scaffold can potentially direct cells to not only restore the integrity but also improve the sound quality recovered for TM perforation patients.
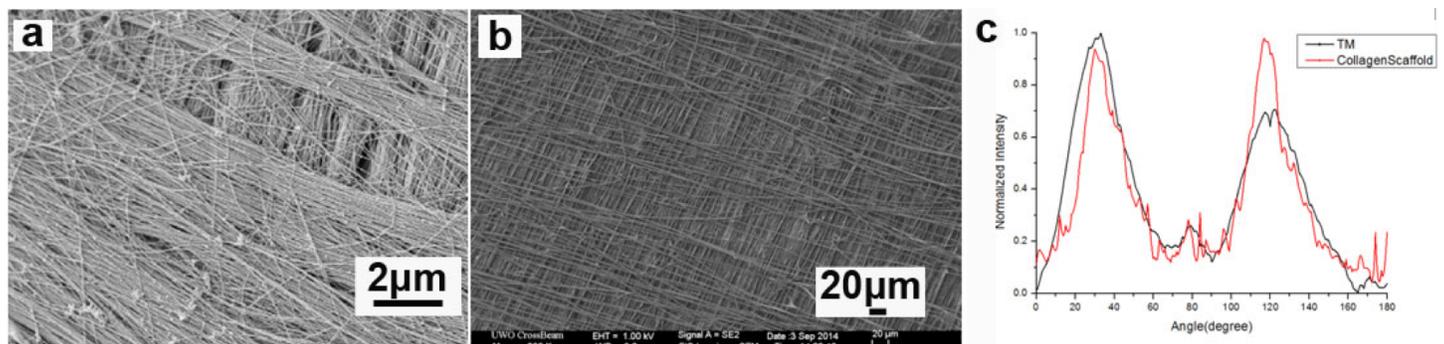
Figure 2: (a) SEM image of rat TM; (b) SEM image of two layers aligned fibers; (c) FFT plot on two layer fibers in figure a and b

1. O'Connor, K.N.; Tam, M.; Blevins, N.H.; Puria, S. Tympanic membrane collagen fibers: a key to high-frequency sound conduction. *Laryngoscope*. **2008**, *118*, 483–490.

2. Liu, J.; Ladak, H.M.; Agrawal, S.K.; Wan, W.K. Fiber arrangement in the rat tympanic membrane. In *36 th Annual Abstract Book of Association for Research in Otolaryngolog Annual Meeting Abstract*. **2013**, *36*, 215-216.

3. Mekhail, M.; Wong, K.K.H.; Padavan, D.T.; Wu, Y.; O'Gorman, D.B.; Wan, W.K. Genipin-cross-linked electrospun collagen fibers. *Journal of Biomaterials Science*. **2010,** *22, 2241-2259*

Surface Analysis for Corrosion Chemistry

Mojtaba Momeni (Ph.D. Candidate), Prof. J.C.Wren
Department of Chemistry,
Western University, London, Ontario, Canada

Corrosion is one of the biggest concerns in modern societies. Ever since humans became dependent upon metals and alloys, the corrosion problem has showed itself. Corrosion, in fact, is the reverse process of metal extraction. By definition, corrosion is a natural process which converts a refined metal to a more stable form, such as its oxide, hydroxide or sulfide. All the elements tend to be found in their most stable condition, one that is dictated by mother nature. For metals, this is mostly in the form of oxides, sulfates. Only a handful of metals such as gold can be found in their metallic form. Nowadays, human's life looks impossible without the use of metals and alloys and this makes the study of corrosion inevitable. Data shows both the direct and indirect cost of corrosion is between 3-5% of gross domestic product (GDP) for most countries. For Canada this comes to a minimum value of more than 60 billion CAD in 2015.

Within an industry such as nuclear energy, addressing corrosion problems is critical to ensuring public safety as well as the safety of the environment. This is important when you take into account that under radioactive conditions, the properties of water (as the corrosive environment) change and the production of highly oxidizing and reducing agents alter the corrosion behaviour significantly.

One of the main methods employed for corrosion control is the use of metals and alloys that can form a protective passive oxide layer on the surface under particular environmental conditions (eg. stainless steels, nickel super alloys…). The formed oxides are able to significantly lower the rate of corrosion and in fact make the material passive. There are many factors that can affect the protectiveness of the oxide layer. Among them are the chemistry of the oxide, its thickness as well as its surface morphology, all of which can change within the environment of corrosion. To study these factors, employing surface analysis methods are crucial. One of the methods that is very helpful is electron microscopy. This technique shows the change in the morphology of the oxide and any possibility of localized corrosion. When coupled with Energy Dispersive X-Ray Spectroscopy (EDX), determination the oxide chemistry is possible. With state-of-art instrumentation, the thickness of the oxide can be determined by Focused Ion Beam (FIB). The figures below show an example of a corroded surface on carbon steel and its measured thickness. This analysis was performed at the Western Nanofabrication Facility at the Western University.
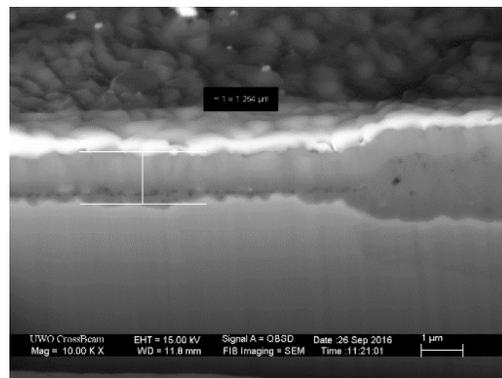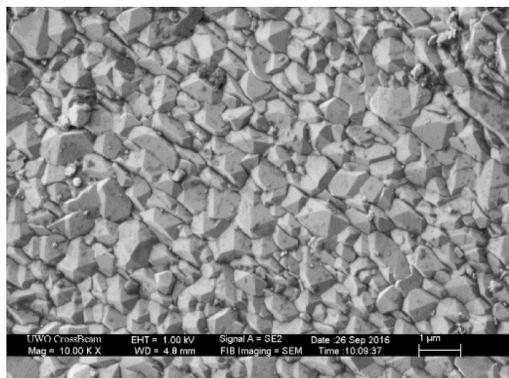
The figure shows the morphology (left) and thickness (right) of the oxide that forms on the steel surface

Superimposed Arrays of Nanoprisms for Multispectral Molecular Plasmonics

Gregory Wallace (Ph.D. Candidate), Prof. François Lagugné-Labarthet
Department of Chemistry,
Western University, London, Ontario, Canada

Collaborators: Dr. Scott M. Rosendahl (Canadian Light Source Inc., Saskatoon, SK, Canada), Dr. Alexandre Merlen (Institut des Matériaux et Nanomatériaux de Provence, Université de Toulon, Toulon Cedex 9, France), Todd W. Simpson (Western Nanofabrication Facility)

The design and fabrication of conductive nanostructures that are capable of yielding a large local electromagnetic enhancement has become a field of great interest. When a molecule is located at or near the surface of the nanostructure and is probed by light, the vibrational fingerprint of the molecule is enhanced. This field of research is known as molecular plasmonics. Although various spectroscopic techniques benefit from this enhancement, performing different spectroscopic measurements on the same platform remains a significant challenge. Especially when these techniques require the use of light in different spectral regions.

Using the Western Nanofabrication Facility, we fabricated a series of platforms comprised of superimposed arrays of gold nanoprisms. We first prepared the superimposed nanoprisms using nanosphere lithography, first with spheres of 1 µm diameter, followed by 6 µm diameter spheres. To gain better control over the size and configuration of the nanoprisms, we further prepared the platforms by electron beam lithography, as shown in Figure A. Here the small nanoprisms were fabricated with a side length of 0.25 µm, along with larger nanoprisms with side lengths varying from 1-2 µm. The aim of these platforms was to generate localized surface plasmon resonances (LSPRs) in the smaller nanoprisms using visible light, and LSPRs in the larger nanoprisms using near and mid-infrared light.

To verify that the LSPRs were in fact in the correct spectral regions, we performed visible and near-infrared absorption measurements in the Lagugné-Labarthet lab, along with near- and mid-infrared absorption measurements on the mid-infrared beamline at the Canadian Light Source (CLS) in Saskatoon, Saskatchewan. To correlate the spectral resonances with their spatial positions, we relied on the use of Finite Difference Time Domain calculations. Based on these results, we believed that the resulting platforms would be compatible with measurements for surface-enhanced fluorescence (SEF), surface-enhanced Raman spectroscopy (SERS), and surface-enhanced infrared absorption (SEIRA).

In order to evaluate the compatibility of the platforms with each of the techniques, platforms were functionalized with either a fluorescence probe (cyanine 5 with a 5000 Dalton polyethylene glycol chain and a thiol, Cy5-PEG-SH) or with a Raman/IR reporter (4-nitrothiophenol, 4-NTP). The SEF results in Figure B demonstrate well that the central regions of the platform, the area with the smaller nanoprisms, do indeed enhance the fluorescence signal, as indicated by the bright colour. Whereas the larger nanoprisms are darker in colour, highlighting that the larger nanoprisms do not provide significant enhancement in the visible spectral region. Similarly, the SERS map in Figure C indicates the same general trend. In the visible spectral region, the smaller nanoprisms yield a greater enhancement than the larger nanoprisms.
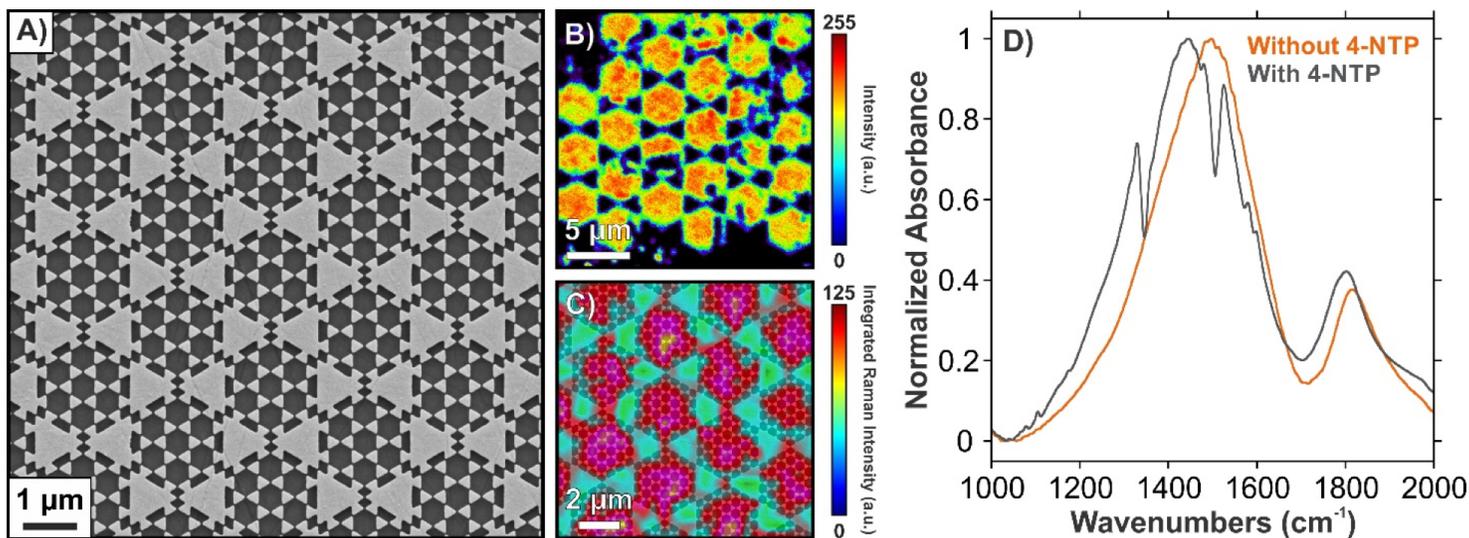
Figure. A) SEM image of arrays of superimposed nanoprisms with side lengths of 0.25 and 1 μm. B) SEF image of a Cy5-PEG-SH functionalized platform. C) SERS map of a 4-NTP functionalized platform integrated the symmetric NO_2 peak from 1300-1350 cm^{-1}. For clarity on the position of the small and large nanoprisms, the image overlaid onto an SEM image of the platform. D) Infrared and SEIRA spectra of a platform with and without functionalization with 4-NTP.

Lastly, we performed SEIRA measurements at the CLS on the same samples that we performed the SERS measurements on. The infrared and SEIRA measurements show that once the surface is functionalized, a noticeable red-shift is observed. Furthermore, we are able to detect the symmetric and asymmetric NO_2 stretches of 4-NTP (shown as decreases in absorbance).

Overall, our developed platform comprised of superimposed nanoprisms offers a simple means of achieving multispectral compatibility. By fabricating the platforms by electron beam lithography as opposed to nanosphere lithography, we were capable of controlling the size, gap size, and configuration of the nanoprisms. As predicted, the resulting platforms exhibit plasmon resonances across the visible and infrared spectral regions, enabling us to perform SEF, SERS, and SEIRA measurements, all on the same platform. In the future, we envision the fabrication of other structures that exhibit multispectral compatibility, along with the integration of such structures into microfluidic channels. In doing so, we plan on using such a complete device for plasmonic sensing of analytes, with a particular emphasis on aptamer-based sensing of small molecules.

Reference: "Superimposed Arrays of Nanoprisms for Multispectral Molecular Plasmonics" ,
Gregory Q. Wallace, M. Tabatabaei, R.Hou, M.J. Coady, P.R. Norton, T.S. Simpson, S.M. Rosendahl,
A.Merlen, and F. Lagugné-Labarthet*, ACS Photonics, 2016, 3, 1723-1732.

Western Nanofabrication Facility

Western University
Physics and Astronomy Building Room 14
London, Ontario N6A 3K7

nanofab.uwo.ca

Prof. François Lagugné-Labarthet
Facility Director
flagugne@uwo.ca

Todd Simpson Ph.D.
Senior Research Scientist
tsimpson@uwo.ca

Tim Goldhawk
Laboratory Supervisor
tim.goldhawk@uwo.ca

Western Science