A Separation and Characteristics of Inner Egg Shell Membrane

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Abstract—The aim of this work is to characterize the inner egg shell membrane (IESM) and compare different methods of extracting it from an egg. Scanning electron microscopy (SEM) was used in a combination with energy dispersive X-Ray spectroscopy (EDX) to get an idea of membrane structure and chemical composition and impacts of the used extraction method. Significant differences were found between the measured samples, but it has not been verified whether they affect the resulting physical properties yet. The importance of this study lies mainly in the future investigation of several factors, such as piezoelectric properties, so it serves as a fundamental research.

Keywords—Egg Shell Membrane, IESM, SEM, EDX, characterization, membranes, extraction

1. INTRODUCTION

Due to a huge development in technology during last years, there is a growing need to think more and more about materials which are used to create our devices. Some materials are becoming more difficult to obtain – their extraction can be devastating for nature or expensive. Humans need to focus more on renewable and biodegradable resources. The inner egg shell membrane (IESM) is naturally piezoelectric [1] and as a result can be used to create high-tech products, for example, pressure sensors.

1.1. Inner egg shell membrane (IESM)

An egg is basically a shelter for a fetus, it contains necessary nutrients for embryonic development and provides protection to it. There are two membranes under the egg shell called inner and outer, their roles are to reinforce fragile shell and significantly increase its anti-bacterial protection [2]. This paper focuses mainly on the inner egg shell membrane due to its piezoelectric properties and greater potential for technological applications.

2. MATERIALS AND METHODS

2.1. Materials

Both hen eggs used for this observation and the cheap distilled vinegar used for membrane separation were purchased from a local supermarket, all eggs were medium size. Pure acetic acid solution (Merci, Brno, Czech Republic) served as an alternative to the vinegar. Deionized water (Merci, Brno, Czech Republic) was used to clean the extracted specimens before observation.

2.2. Membrane separation

In order to separate the membrane from the rest of the egg, various techniques have been tried. Subsequently, all samples have been compared to determine which of the methods least affected the desired properties.

At first, membrane was isolated directly from a cracked egg. It is not very hard, but it is not possible to acquire larger parts intact. The samples obtained this way were only several square centimeters in size on average.

Another way to easily separate membrane is to chemically decompose the shell. The cheap distilled vinegar was used as the first chemical solvent. It lasted about two days to fully decompose the outer shell with vinegar. In addition, for closer examination of this method two concentrations of acetic acid solution were used. In widely available kitchen vinegar concentration of acetic acid is between 4 and 18 % and further used concentrations were 30 % and 90 %.

All methods mentioned above have been tried both on boiled and unboiled eggs. After separating the membrane, all specimens were washed in deionized water, placed on SEM stab and dried at the temperature of 60 ˚C for one hour. They were also coated by 7 nm of gold using the EM ACE600 (Leica, Wetzlar, Germany) coater to prepare them for SEM observation. Gold coating was preferred over carbon coating for the purpose of following EDX analysis.

2.3. Specimens measurements and observation

All specimens were observed using Lyra3 SEM (Tescan, Brno, Czech Republic). Because the membrane is a biological material, only 5 kV was used as the acceleration voltage, higher voltage could possibly damage a membrane structure. A total of 5 samples were observed from both top and bottom side. Specimens are fully described in Table I.

Different parts of the membrane from multiple specimens were also exposed to EDX analysis using X-Max 50 device (Oxford Instruments, Abingdon-on-Thames, Great Britain), acceleration voltage for the EDX was 10 kV and the measurement lasted two hours.

Table I: Specimens description.

Label	IESM separation method
S1	boiled egg in vinegar
S2	unboiled egg in vinegar
S3	directly from cracked egg (unboiled)
S4	boiled egg in 30 % acetic acid solution
S5	boiled egg in 90 % acetic acid solution

3. RESULTS

3.1. Scanning electron microscopy (SEM)

After the SEM observation of the first round of specimens, significant differences between the samples were observed, indicating that the membrane has two different looking sides. This assumption was confirmed by the observation of an additional set of specimens showing both sides of the same membrane sample. As Fig. 1 shows, the specimen S3 was torn into two pieces, which allowed us to observe both sides of the membrane simultaneously.

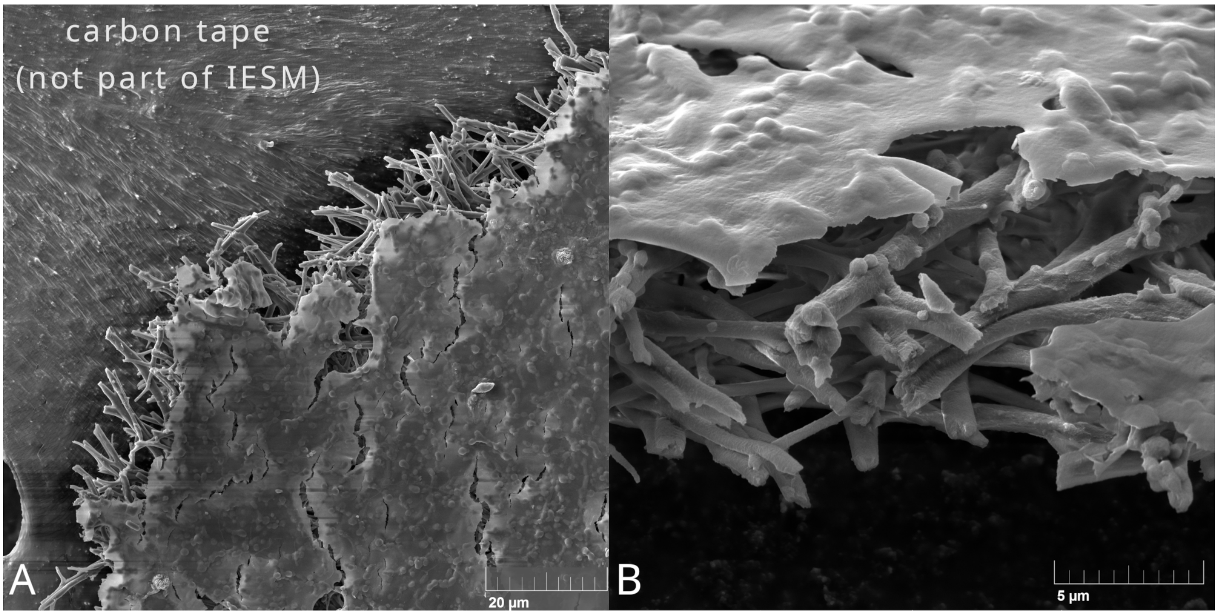

Figure 1: The torn piece (S3) clearly shows that the two sides of the membrane are different. (A) is a view from the top with carbon tape in the background, (B) is a cross-section image from 50° angle.

One side of the membrane is formed by highly porous fiber network while the other side is rather solid. As Fig. 1B shows, the more solid side is significantly thinner. From a simple calculation, the thickness of the membrane at this spot was estimated to be 8 µm. This value is not corresponding to the information provided by M. U. Khan, Q. M. Saqib and G. Hassan in their articles [3, 4] which was about 20 µm. Also, a different specimen was measured on the cleanly cut edge instead of the torn one and the observed value was about 40 µm. The thickness varies greatly and could depend on the age and size of the egg, different kinds of hens and other factors as well. To specify the values, it would be necessary to conduct further research. A detailed view of the membrane fiber side is shown on Fig. 2C and D.

All specimens were carefully observed using SEM and a few significant differences were found. On some specimens, a fiber structure was perfectly visible and fibers looked clear and compact. On the other hand, some specimens contained regularly dispersed viscous structures around 20 µm in size and a viscous cover was also on most of the fibers and between them. These differences are depicted in Fig. 2A and B. Some differences were also found on the solid side of the membrane, mainly the variation in surface roughness.

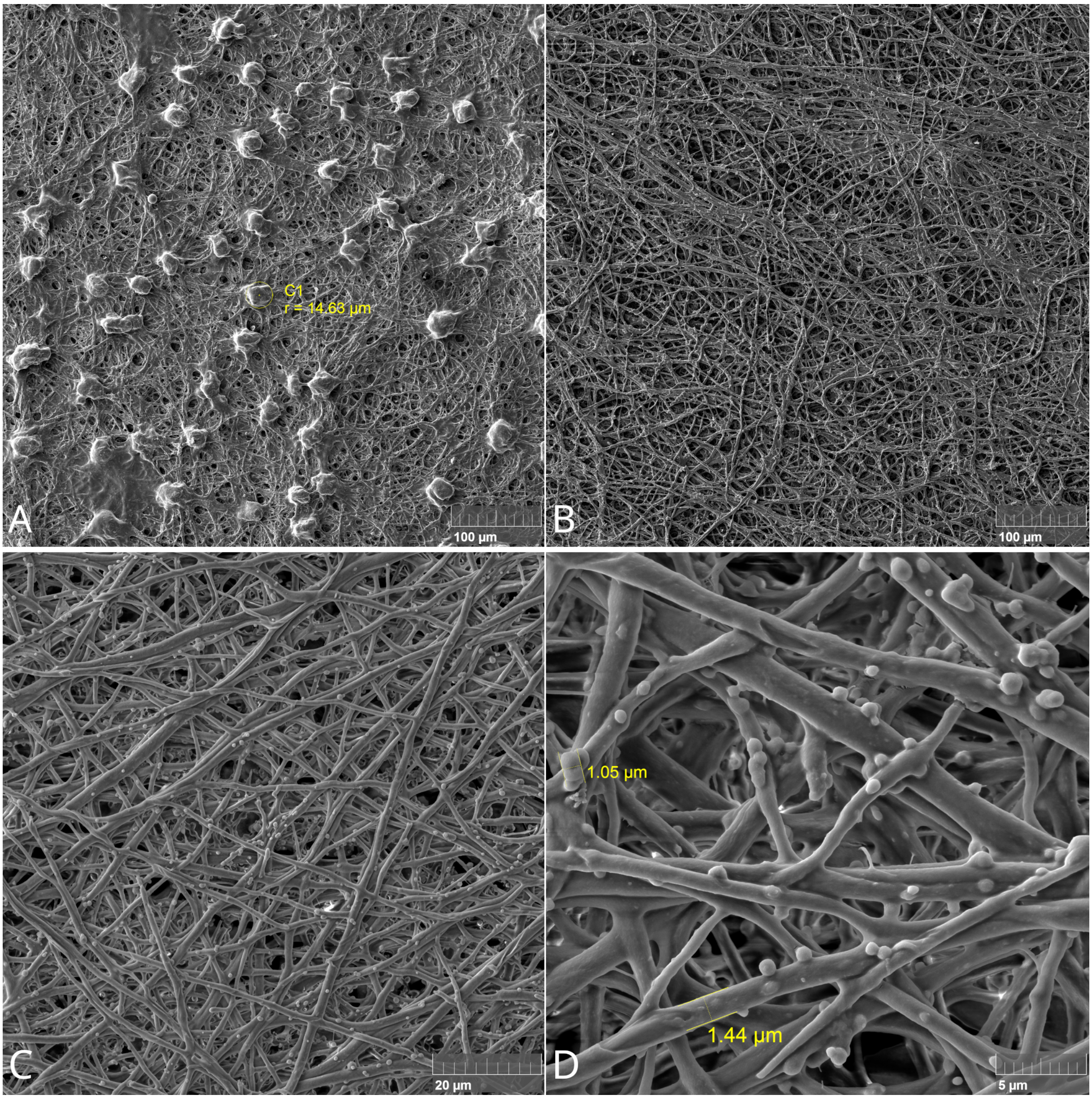

Figure 2: Difference between S2 (A) a S3 (B); More detailed images of S1 structure (C and D).

3.2. Energy dispersive X-Ray spectroscopy (EDX)

Both sides of the membrane were processed by EDX analysis to find their chemical composition. No significant differences were found and it was confirmed that the chemical composition of all investigated samples is very similar. The results also correspond with the articles mentioned above [3, 4]. On 350 eV there is probably a calcium peak hidden, interfering with the peak of carbon. Due to this it is difficult to determine the percentage distribution of the elements. The Fig. 3 contains the most interesting part of the EDX representation of the S5 specimen.

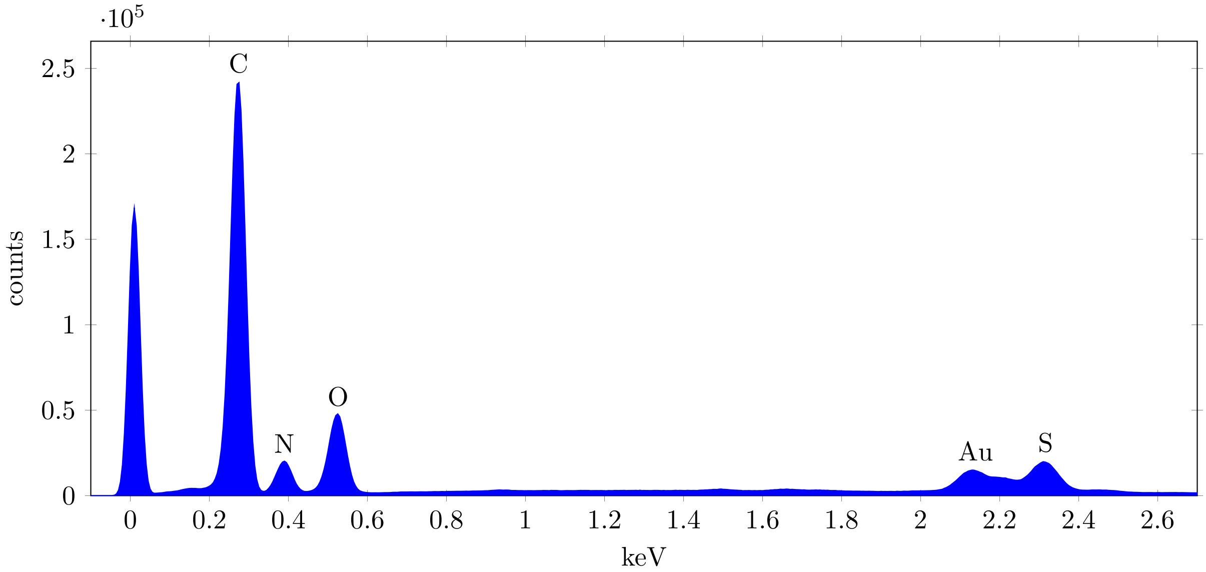

Figure 3: A part of the EDX representation of S5 (10 kV).

4. CONCLUSION

The paper describes the process of IESM characterization and several methods of extraction a membrane from an egg. Using SEM and EDX analysis, it was found that all used methods could be applied for membrane separation, but significant differences between observed specimens were found. The future study is needed to determine whether these anomalies have any impact on desired physical properties with main scope on piezoelectricity. Finding an effect of egg age to the IESM structure or properties would also be very useful.

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