SURFACE MORPHOLOGY OF BIOCERAMIC BOVINE BONE AND FREEZE DRIED BOVINE PERICARDIUM BY ELECTRON MICROSCOPE AND INFINITE FOCUS MEASUREMENT: A COMPARATIVE STUDY

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The challenge to get more information in the observation of surface morphology on biomaterial by using electron and optical microscope are increased by the year. The aim of this study was to compare the imaging methods used in surface morphology observation of bioceramic bovine bone and freeze dried bovine pericardium. The bioceramic bovine bone and freeze dried bovine pericardium were observed by Tabletop Microscope (TM), Field Emission Scanning Electron Microscope (FESEM) and Infinite Focus Measurement device (IFM). For FESEM, the samples were coated with gold. Observations using the FESEM on bioceramic bovine bone and freeze dried bovine pericardium showed improved imaging quality with respect to contrast and charging effect. More detailed information on porous structure of the bioceramic bovine bone and collagen architecture of the freeze dried bovine pericardium were acquired. The TM using low voltage and high chamber pressure showed better quality images in the case of severely charging effect. The small depth of focus of an optical system with vertical scanning in the IFM provided information on surface texture of roughness. In conclusion, the combination of the FESEM and the IFM in the microscopic observation of the bioceramic bovine bone and the freeze dried bovine pericardium is able to provide better quality imaging with detailed information on the structure and surface texture.

Keywords: Surface Morphology, Bioceramic Bovine Bone, Freeze Dried Bovine Pericardium, Electron Microscope, Infinite Focus measurement

INTRODUCTION

The challenge to get more information on surface morphology of biomaterial samples by using electron and optical microscope are increased by the year. Characterization of the surface morphology of the biomaterial becomes very important to determine the success or failure of the biomaterial [1-3]. The surface properties that are of common interest in biomaterial studies include morphology (domain structure), topography (surface roughness), chemical structure, hydrophilicity or hydrophobicity and the presence of ionic groups [2-4,8]. Scanning electron microscope (SEM), transmission electron microscope (TEM), atomic force microscope (AFM), scanning tunneling microscopy (STM), infinite focus measurement machine (IFM) and confocal laser scanning microscopy are microscopic techniques that always are mentioned in the literature of biomaterials.

Bioceramic bovine bone and freeze dried bovine pericardium are well known as implantable tissue grafts. Bioceramic bovine bone is widely used as bone tissue replacement while freeze dried bovine pericardium is normally used as soft tissue replacement in internal body defect and heart valve reconstruction [5, 6]. The surface properties of these biomaterials are essential in correlating any effects of surface modification by processing with changes in biological performance [3].

Appropriate microscopy analysis method will provide desired observation, thus giving better information on surface morphology. Hence, the right decision must be made when planning an experiment. Merret *et. al* (2002) realized that in surface analysis, significant costs associated with sample processing and preparation, lengthy time usage of the equipment and technician time become the main constraint that affect the choice of surface analysis method to be used. However, early information on suitable surface method analysis upon the subject test certainly can minimize the constraint.

In this study, surface characterizations of bioceramic bovine bone and freeze-dried bovine pericardium were observed and analyzed by using optical microscope which is Infinite Focus Measurement device and electron beam microscope which are Tabletop Microscope (TM) and Field Emission Scanning Electron Microscope (FESEM). The paper provides an overview by comparing the three imaging methods currently used in surface properties of bioceramic bovine bone and freeze dried bovine pericardium.

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METHODS AND MATERIALS

Surface morphology was examined with HITACHI SU8000 ultra high resolution field emission scanning electron microscope (FESEM) and HITACHI TM-3000 tabletop scanning electron microscope (TM). To reduce the charging effect, some samples from freeze dried bovine pericardium were gold coated. Then, samples were stacked on the carbon tape and placed on the designated stage. The carbon tapes act as an electrical conductivity between the sample and the stage. Surface topography was examined with an Infinite Focus Measurement (IFM) device, Alicona, 3D optical microscope. Samples were placed onto the motorized stage. The micrograph resolutions and data that could be achieved were the main concern in our study. Comparison was made on bioceramic bovine bone and freeze-dried bovine pericardium with the same magnification captured by the instruments.

For bioceramic bovine bone, the cancellous part of the bovine femoral head were cleaned and cut into 10 mm x 10 mm x 10 mm pieces. The bones were sintered at 800 ^{o}C using Carbolite furnace. Three samples of bioceramic bone were used for each microscopy analysis. For freeze dried bovine pericardium, the pericardium was obtained from an abattoir and trimmed into sheets after the removal of adherent fat. The pericardium was cleaned and disinfected in 0.05% sodium hypochlorite. Freeze-drying was performed using a LyoLab, Lyophilization System Inc, USA freeze dryer. After the freeze drying process, the freeze dried bovine pericardium was cut into 10mm x 10 mm pieces. Three samples of freeze dried bovine pericardium were used for each microscopy analysis.

RESULTS AND DISCUSSION

A number of researchers that studied on freeze dried bovine pericardium and bioceramic bovine bone intended to use non destructive technique without inhibiting the ability to obtain the desired information. The information about the surface properties plays a crucial role on the interface between implants and surrounding host tissues [9]. In the present study, the emphasis was not on describing the morphological and structural properties of the biomaterials, but rather to overview the microscopic methods used to acquire the best micrograph.

Observations using FESEM on bioceramic bovine bone and freeze dried bovine pericardium produced better imaging quality with respect to contrast and charging effect as compared to TM and IFM. High brightness cold field emission gun in the FESEM technology is very useful for the observation at ultrahigh resolution and high sensitivity [10]. Fig. 1(a) seems to have high resolution compared to Fig. 1(b). With beam deceleration technology in FESEM, the images performance improved although at very low accelerating voltage [10]. At low voltage, details of membranes surface produce visible contrast and the surface details become progressively more transparent with an increase in beam energy because of beam penetration through the structure [11]. Many subtle objects can be routinely seen on the FESEM micrographs. Thus, more detailed information of porous structure in the bovine bioceramic and collagen architecture in the freeze dried bovine pericardium were acquired.

FESEM acquisition image can be done fairly quickly allowing for a large number of images to be obtained with varying magnifications. In addition to imaging the surface morphology of bioceramic, FESEM is normally combined with X-ray analysis (EDX) to determine elemental distribution. EDX determines the elements of the bulk material rather than the surfaces as the sampling depth is in the order of micrometers [11]. TM is a compact, simple to use and variable pressure SEM. It can be considered as a perfect imaging platform bridging the gap between light microscopy and conventional SEM [13]. TM presents stereoscopic detail micrograph by using the annual multi-segmented detector and shows contrast from variances in the atomic number composition of the sample, able to observe topographical details of sample [12]. It provides greater flexibility in imaging and analysis of samples by selection between dual accelerating voltage which is between 5 kV and 15 kV. The selection of 5kV enhances the surface structure and optimizes imaging on soft materials while 15 kV allows high resolution topographical and compositional imaging [12]. TM has advantages for imaging non conductive samples in low magnification range which gives a large view area and sharp image with high depth of focus. Thus make it suitable for investigating the biological samples.

Since the TM uses low voltage and high chamber pressure it shows the better quality images in the case of severely charging. The ability to visualize low-contrast objects is the essence of good contrast in TM. Micrograph observed from TM seemed to have less resolution when compared to FESEM. For the freeze dried bovine pericardium, TM micrograph at 5 kV accelerating voltage (Fig.2b) has high contrast and accumulation of static electric charges on the surface which obscure the detail. On the other hand, FESEM micrograph gathered at 1 kV accelerating voltage appears to give good contrast

and high resolution. Furthermore, TM can be equipped with the optional EDS/EDX analysis of sample which is producing elemental composition mapping data with automatic reporting when used at 15 kV.

IFM is the optical 3D measurement instrument that simultaneously captures the entire surface topographic information in combination with its true color information [7, 12]. The resulting image of IFM is similar to conventional light microscope in a way that it shows limited depth of focus and measure the surface roughness of the samples [7]. The IFM can generally be considered as complementary technique in the surface analysis and useful in cases where only imaging and measurement is required [12].

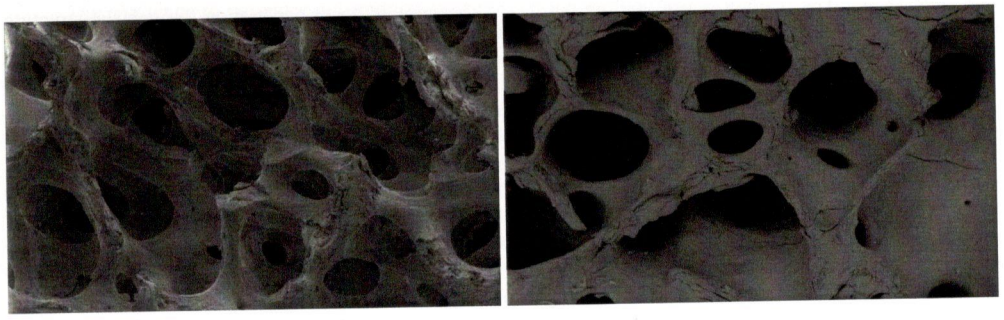


Fig.1. The porous structure of bioceramic bovine bone observed from FESEM (Fig.1a) and TM (Fig.1b) at 15 kV with 60x magnification

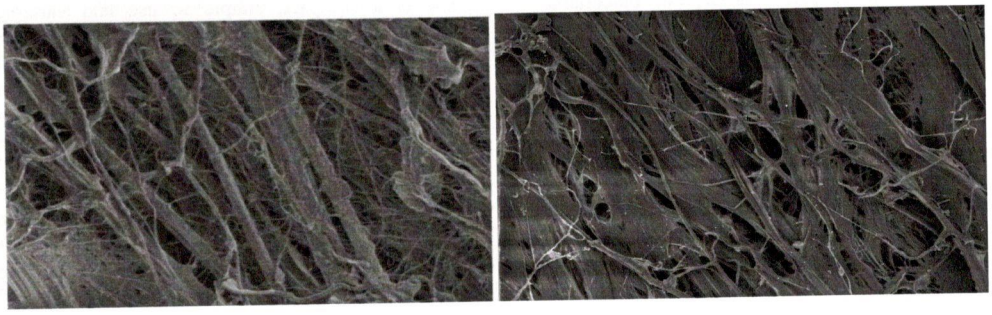


Fig.2. The collagen architectures of freeze dried bovine pericardium observed from FESEM (Fig.2a) at 1 kV and TM (Fig.2b) at 5 kV with 150x magnification.

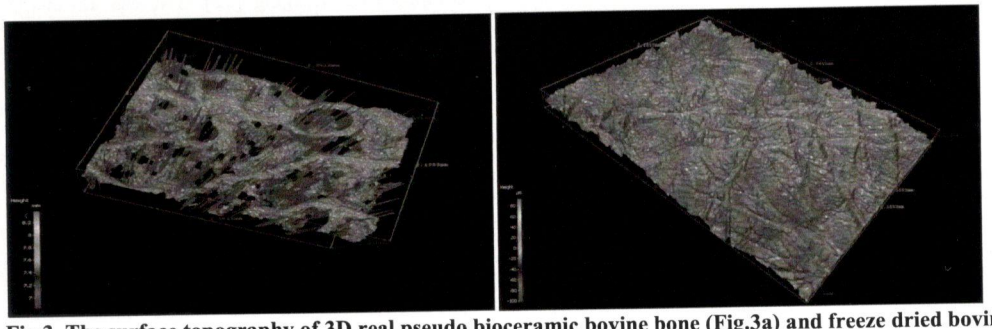


Fig.3. The surface topography of 3D real pseudo bioceramic bovine bone (Fig.3a) and freeze dried bovine pericardium (Fig.3b) at 10x magnification results from IFM.

The small depth of focus of and optical system with vertical scanning in IFM provide surface texture of roughness information. Using the 3 D measurement device, it gives information on real optical graphic by analyzing the surface texture of roughness. In Fig.3(a) the 3D real pseudo bioceramic bovine bone shows some peaks which determine the data loss from the complexity of porous structure. From this observation, bioceramic bovine bone was not possible to be measured with IFM since surface is inhomogeneous and has a shadow.

Lorenzo (2010) described the general advantages and disadvantages of the optical methods in his thesis. The advantages are non-contact technique which has special applications regarding soft material, the possibility of reaching difficult points especially when the measuring object has very small dimension and acquisition of more data in less time. The disadvantages of the optical methods are the difficulties in measuring deep cavities or pores, limitation of the working distance and the quality of the lenses. This study indicated the limitation of using IFM on bioceramic bovine bone.

Microscopic observations on the bioceramic bovine bone as in Fig.1(a) and Fig.1(b) show the elimination of organic compound precipitate on the porous structure. The porous structure is still retained and production of hydroxyapatite (HA) is clearly determined by the energy dispersive X-ray analysis (EDX) using FESEM. The topography structure of the bioceramic bovine bone as in Fig.3(a) is indistinctly achieved due to the deep pores and complexity structure that affect the optical reflection. Bovine pericardium is mainly fibro-collagenous in nature with few cellular and vascular elements. Freeze-drying preservation method causes the effects on ultrastructural of the grafts. The collagen architecture of freeze dried bovine pericardium remains intact as observed in Fig.2(a) and 2(b) while the topography structure in Fig.3(b) clearly describes the surface roughness.

Nevertheless, surface is very important in membrane processing since the surface determines the performance of the membrane. Biological material normally is poorly conductive. FESEM has been shown to be a powerful investigative tool in examining surface morphology in membrane research. A significant advance has been made recently by means of FESEM. Fig.1(a) shows high resolution micrograph of the bioceramic bovine bone by FESEM at 15 kV. The images are outstanding not only for their resolution and contrast but also for the visible details of pore structure. Compared with images by FESEM, TM and optical IFM, the FESEM micrographs give distinct and detailed views of the membrane surface and its morphology.

CONCLUSIONS

This paper provides an overview of three microscopic techniques commonly used in biomaterials, particularly bioceramic bovine bone and freeze dried bovine pericardium. A combination of the surface analysis methods is crucial to provide the inclusive information necessary for correlating surface morphology with performance. In this study, FESEM in combination with IFM provide more information. However, for investigating bioceramic bovine bone either FESEM or TM can be used. If the cost is the hurdle, the TM can be the alternative. In future, a comparative study can be extended to other microscopic instruments.

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