Innovative research of plasma physics for life sciences

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Abstract. In medicine, cold atmospheric plasma (CAP) for the medical treatment is a new field in plasma application, called plasma medicine. CAP contains mix of excited atoms and molecules, UV photons, charged particles as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Typical species in air-CAPs are O_3, OH, N_xO_x, and HNO_x. The current developments in this field have fuelled the hope that CAP could be an interesting new therapeutic approach in the treatment of cancer. CAP apparently demonstrated effect on cancer cell apoptosis which did not induce cell necrosis or disruption. Moreover, CAP seemed to be selective for cancer cells since it was more effective in tumor cells than in normal non-neoplastic cells. In bioscience, dentistry and veterinary medicine : Since CAP, is delivered at room temperature, which results in less damaging effects on living tissue, while still has the efficiency in disinfection and sterilization. Recent studies proved that it is able to inactivate gram-negative and gram-positive bacteria, fungi, virus, spore, various parasites, and foreign organisms or pathogens without harming tissue. Moreover, cold plasma has been used effectively in medical field such as dental use, inducing apoptosis of malignant cells, stopping bleeding, promoting wound healing and tissue regeneration. Sericin hydrolysates, originating from silkworm is found support cell proliferation, expand cell adhesion and increase cell yield. The covalent linkage between a bioactive protein molecule and polystyrene dish surface via a carbon intermediate layer can slow down the release rate of protein compound into the phosphate buffer saline (PBS) solution. We found that a-C films and a-C:N_2 films show good attachment of human bone marrow-derived mesenchymal stem cells (hBM-MSCs). All of carbon modified-Polystyrene(PS) dishes revealed the less release rate of sericin molecules into PBS solution than PS control.

1. Introduction

In term of physics and chemistry, plasma is a completely or partially ionized gas with ions, electrons and uncharged particles such as atoms, molecules, and radicals, regarded as fourth state of matter. The other states of matter are liquid, gas, and solid. Plasma does not have a definite shape or volume as gas; however, it will form structures when put under a magnetic field, which is not seen in gas. From a macroscopic point of view, plasma is electrically neutral. However, plasma is electrically conductive and a lot of free charge carriers are contained in it.

Depending on amounts of energy transferred, the plasma properties change in terms of electron density and electron temperature. These two parameters define plasmas into two categories: thermal equilibrium plasma or thermal plasma and non-thermal equilibrium plasma or cold plasma.

• Thermal plasma, transitions and chemical reactions are mainly controlled by plasma particles collisions, not by radiative processes. Moreover, collision phenomena are micro-reversible in thermal plasma, suggesting that each kind of collision is balanced by its inverse such as excitation/de-

excitation; ionization/recombination; kinetic balance [1]. Therefore, in thermal plasma the plasma temperature is equal to the gas temperature.

• Cold plasma, described by two temperatures: electron temperature (T_e) and ion temperature (T_i). Because of the large mass difference between electrons and ions, the plasma temperature or gas temperature is determined by ion temperature. On the other hand, the electron-induced de-excitation rate of the atom is generally lower than the corresponding electron-induced excitation rate because of a significant radiative de-excitation rate. Therefore, the density distribution of excited atoms in cold plasma is possible to depart from Boltzmann distribution, saying that the plasma temperature is much lower than the electron temperature [2-5].

2. Plasma for application in life sciences

2.1. The cold atmospheric plasma (CAP)

There are many different types of cold plasma sources for bio- or life-science applications. However, dielectric barrier discharge (DBD) atmospheric pressure, room air plasma has great potential for aesthetic medicine. In his report, Fridman et al. has confidently concluded that the direct plasma or filamentary DBD is much more potent in bacterial eradication than the indirect plasma or glow DBD type [6-8]. Nevertheless, the direct plasma source using the DBD technique can work only at very small millimeters distance, the gap between the powered or floating electrode and skin as second or ground electrode. The problem with using a DBD electrode on a non uniform skin surface is that the geometry and variation of skin electrical properties make it difficult to apply the flat planar floating electrode clinically. It is difficult to maintain and fix small-millimeter gap distances to create a uniform and stable homogeneous plasma beam without uncontrollable streamer discharge. A novel CAP device operated in mixed filamentary and glow discharge using the FE-DBD configuration has been developed by Photo Bio Care, Thailand as shown schematically in Fig. 1. The device can create a low-temperature, sustained, homogeneous micro-filament beam (23) that emits from the, round shape designed, dielectric floating electrode (22) when it comes in direct contact with a dry or mildly moist skin surface; e.g. wounds (24). The round shape of the dielectric electrode tip can be varied from a few millimeters up to 25 mm. The electrode used in this study is 18 mm in diameter. Such geometry creates regular, small gap distances once the electrode is mildly pressed to the skin surface and is moved constantly.

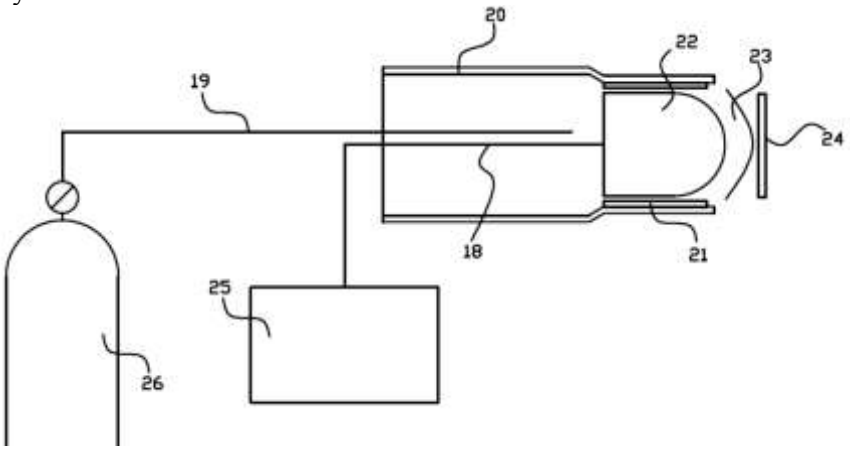

Fig. 1. A FE-DBD plasma device developed for bioscience applications.

The power generator (25) drives high-frequency pulse waves in the range of 15–20 KHz. Peak-to-peak voltage is ~6–7 kV, delivered as pulsing and adjustable rate from 10 to 110 Hz. The maximum in-put power from a normal household socket is 40 watts. Power intensity is adjustable from 1 to 10. The plasma power output is in the range of 0.2 – 1 watt, adjustable according to intensity and repetitive pulse setting. The automatic preset is at level 05 intensity at 50 Hz, with average CAP power of 0.62

watts. The system is set at a duration limit of 20 minutes per treatment. Also, it is equipped with Argon gas (26) to perform a hybrid mode CAP operation.

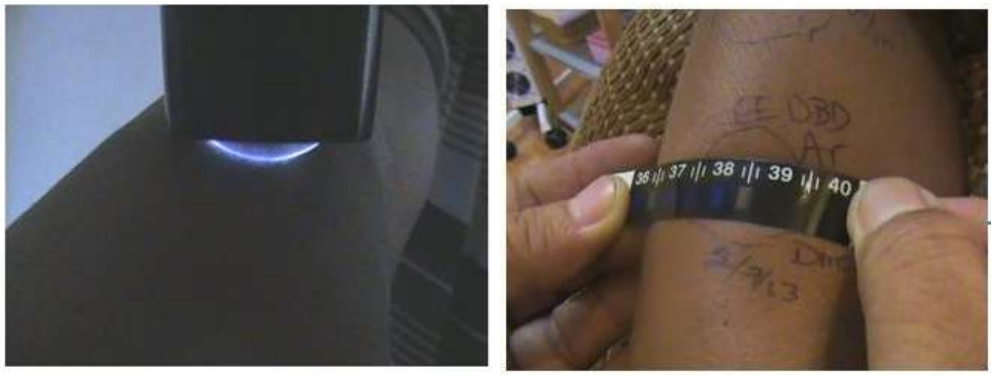

Fig. 2. CAP was applied to arm skin and temperature strip was measured afterward, from left to right. The setting was 5/50 at 24^{o}C 50% RH [9].

Table 1. Skin temperature and sense of power of volunteers with CAP application time [9].

Application time (s)	-	2	3	4	5
Measured temp (^{o}C)	26.5	29.5	32.0	35.5	39.0
Sense of volunteer	-	feel warm	warm	Quite warm	hot

2.1.1. Radicals in the CAP. In CAP, feed or air gas molecules are excited and ionized through the collisions with energetic electrons. And most of them are responding by electron-induced excitation mode in the non-equilibrium plasma. Therefore, the active species such as excited molecules, atoms, and ions in CAP emits light through a significant radiative de-excitation. Optical emission spectroscopy (OES) is well known as a non-invasive and non-disturbing technique for general plasma diagnostics as shown in Fig. 3.

To determine number of radicals particularly in this CAP, the spectroscopy can be rearranged from OES to UV-absorption spectroscopy [10]. And the density of ground state hydroxyl (OH) radicals, could be carried out by UV absorption spectroscopy using the Lambert-Beer's law,

$$[\mathrm{OH}] = -\frac{1}{\sigma \cdot l}\ln\left(\frac{\mathrm{I}^{t+p}-\mathrm{I}^{p}}{\mathrm{I}^{s}}\right) ,$$

where σ is the attenuation cross section of for absorbing species of OH radical, and l is the plasma spatial depth which is the UV beam passed through.

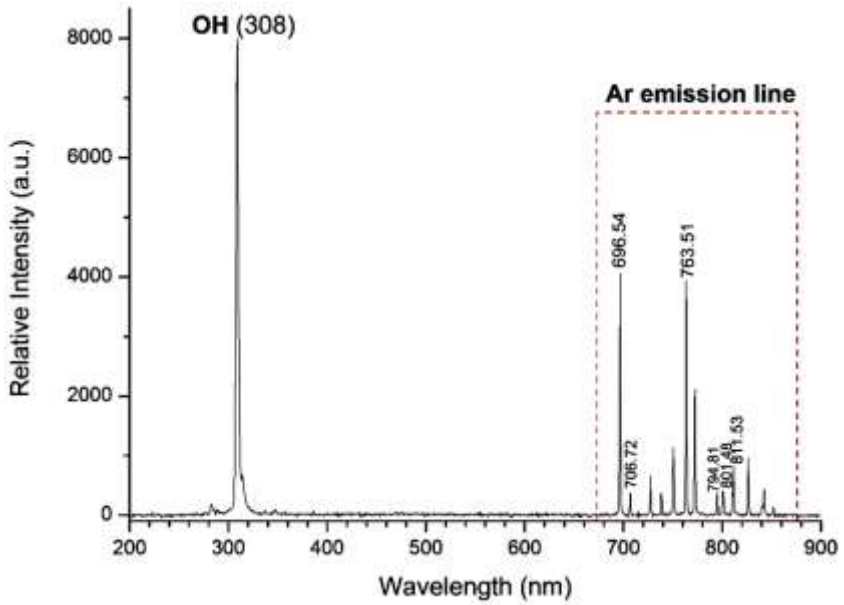

Fig. 3. A typical optical emission spectroscopy of CAP using Argon as a working gas [10].

Whereas I^{t+p} is the total light intensity or the sum of the transmitted light and plasma light measured with UV lamp and plasma on, I^{p} is the plasma intensity with the source off and I^{s} is the intensity of the source with the plasma off. The numbers of OH radical based on the absorbed band head at 306.4 nm from electronic transition $A^2\Sigma \rightarrow X^2\Pi$ has been investigated versus Argon flow rate with some settings of the hybrid CAP and summarized in Table 2.

Table 2. Numbers of OH radical ($x10^{15}$ cm^{-3}) from the hybrid CAP at some settings [10].

Flow / Setting	5/10	5/50	5/110
@ Ar 4.5 lpm	.74	1.8	2.2
@ Ar 5 lpm	.68	1.7	2.0
@ Ar 6 lpm	.42	.76	.95

In a Ar-CAP, the OH radical is primarily ascribed by electrons and Ar metastables (Ar_m) induced by dissociation of water molecules in ambient air. Two dominant mechanisms are: $e^- + H_2O \rightarrow OH + H + e^-$, $k_1 = 2 \times 10^{-18\sim-16}$ m^3 s^{-1} and $Ar_m + H_2O \rightarrow OH + H + Ar$, $k_2 = 4.5 \times 10^{-16}$ m^3 s^{-1} where k_1 is estimated at typical conditions for low temperature plasma, i.e. low ionization degree ($\sim 10^{-4}$), an electron temperature of 1–2 eV.

2.1.2. The efficacy of CAP in bioscience. Through the oxidation process, chemically reactive oxygen species (ROS) are known to be effective in the disruption of the bacterial cell wall [11-13]. The role of oxygen radicals in plasma sterilization has been also demonstrated: the rate of bacterial inactivation in the oxygen plasma group was greater than that in the helium plasma group. This is due to the presence of oxygen-based active species, such as atomic oxygen, the metastable singlet state of oxygen, and

ozone (O_3). Also, in the presence of moisture, the OH radical, is expected to play a significant role by chemically attacking the outer structures of bacterial cells. This role also affirmed by the ATR-FTIR spectra of Methicillin-resistant Staphylococcus aureus (MRSA) specimens in Fig. 4. In gram-negative bacteria like E.coli main mechanism comes from OH radicals form carboxylic acid. In this case OH radical is strong and affect in disrupting an integrity of phospholipids layer (P=O: phosphine oxide) of cell membrane. The effect is clearer when <0.5% oxygen was added and oxygen radicals (O-) enhanced damage via increment of alcohol (C-O).

Scanning electron micrographs in Fig.5 is the instant effect of CAP treatment on *C. albicans* and *E. faecalis* biofilms [9]. It can clearly see that cells wall show ruptures or shrinkage for all over. These un-healthy cells in biofilm are unable to proliferate.

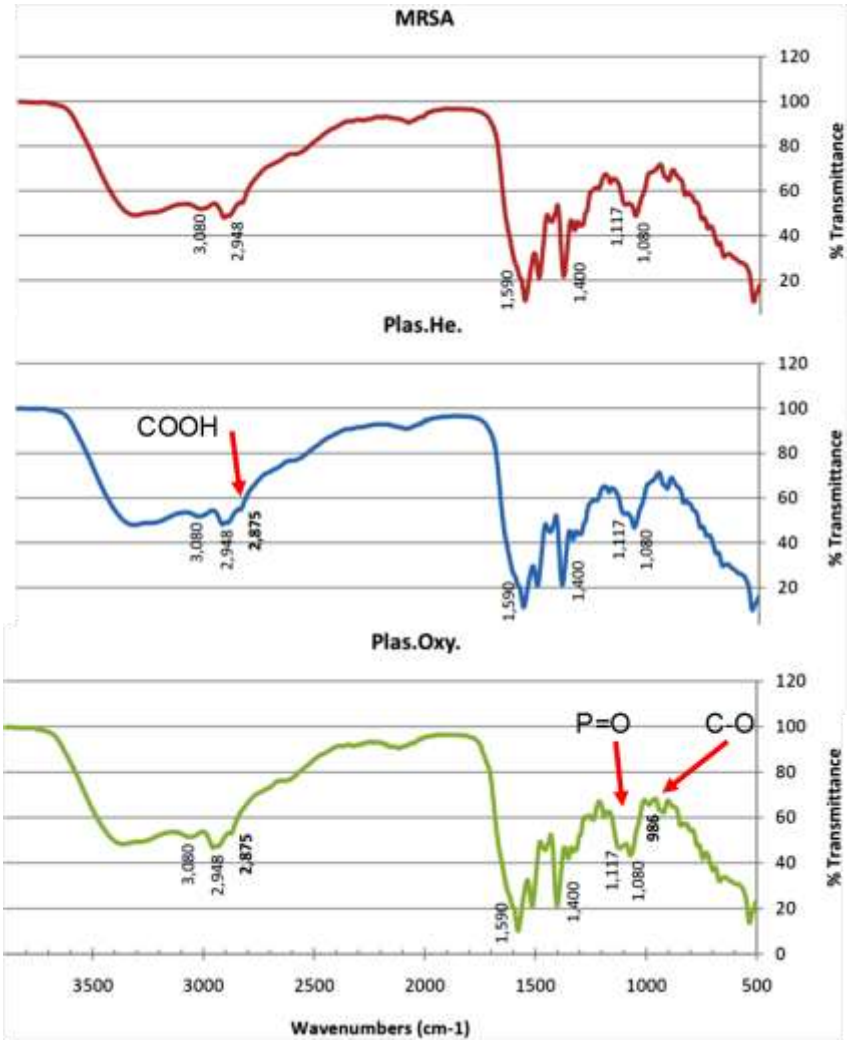

Fig. 4. ATR-FTIR spectra of MRSA specimens revealed reactive species in He/O_2-CAP interact with phospholipids layer.

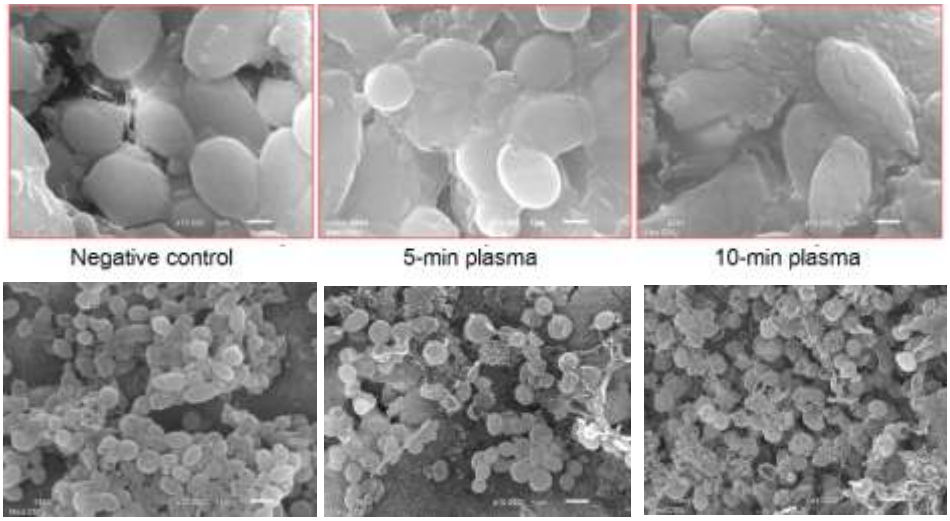

Fig. 5. Instant effect of the CAP treatment on *C.albicans* (top) and *E.faecalis* (bottom) biofilms [9].

2.1.3. Case study: the wound healing. A chronic wound such as bed sore or pressure sore is one of the illness that create a heavy and chronic burden to patient and health care service system. Pressure sores are soft-tissue injuries resulting from an unrelieved pressure of soft tissue over a bony prominence. Most of them will turn to a chronic wound stage with multiple drug resistant bacterial superimposed infections. Such wound usually becomes heavy exudative forming biofilm and necrotic tissue debris. In this study [14] 42 patients were invited to participate if they had chronic pressure ulcer grade 3 or 4. They were divided into 2 groups using block randomized technique. Fig. 6 shows the significantly reduction of bacterial load from wound after the first week by Ar-hybrid CAP treatment compare with standard wound care as control group ($p = 0.001$ for week 1 and 4, $p = 0.002$ for week 8). The plasma treatment was carried out once weekly with 1 min/cm^2 plasma dose.

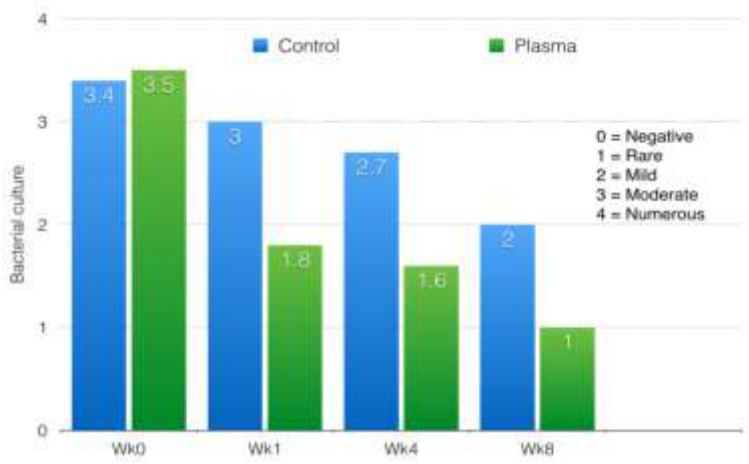

Fig. 6. Bacterial culture reduction between control and CAP treatment group.
($p = 0.001$ for week 1 and 4, $p = 0.002$ for week 8)

Fig. 7 shows the follow-up of wound healing by the plasma treatment. The number of wounds that reduce in size compared to the initial assessment were statistically different at second week after 2 treatments. The number of wounds that reduce in amount of exudate compared to the initial assessment were statistically different at first week after one treatment. The number of wounds that

improve tissue type of wound base compared to the initial assessment were statistically different at second week after 2 treatments. The number of wounds that improve in overall wound healing process compared to the initial assessment were statistically different at first week after one treatment. This in-vivo clinical results may allow the conclusion that CAP is a promising new option for the treatment of chronic wounds.

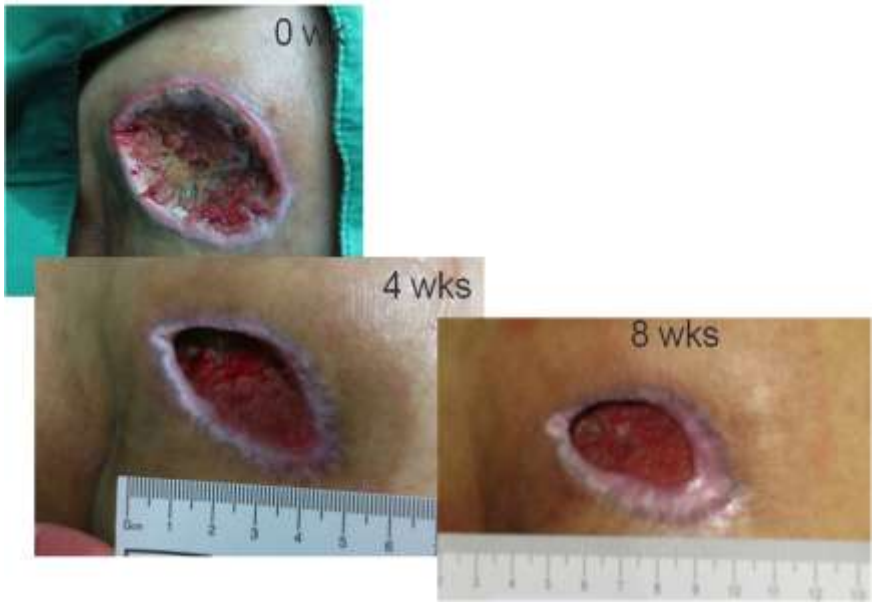

Fig. 7. Photographs of an infected wound for 8 weeks of Ar-hybrid CAP treatment.

2.2. The bioactive molecule layer for biomaterials [15]

2.2.1. We studied the effect of a covalent linkage between a bioactive protein molecule and polystyrene dish surface via a carbon intermediate layer can slow down the release rate of protein compound into the phosphate buffer saline (PBS) solution. Films of amorphous carbon (a-C) and functionalized carbon were deposited on polystyrene (PS) culture dish surfaces using the RF plasma-enhanced chemical vapor deposition (PECVD) system as shown in Fig. 8. Cell attachment and proliferation assay were quantitatively evaluated. Cells were plated at a density of 1×10^4 *cells* per dish with 150 μl of WST-1 reagent (Roche, USA) and incubated at 37^{o}C for 4 *h*. The proliferation assay was analyzed using the OD450 value at day 1 and day 7 in comparison to the control at day 1, which was presented as percentage of attached and proliferated cells.

Fig. 8. RF PECVD system for a-C film synthesis on PS dishes.

The synthesized a-C based-films can increase the hydrophlilicity and biocompatibility of PS dish, especially a-C films and a-C:N_2 films which show the good attachment of human bone marrow-derived mesenchymal stem cells (hBM-MSCs). The carbon-coated PS dishes grafting with sericin protein were used in serum-free condition. We also found that hBM-MSCs have higher % of proliferated cells at day 7 for the modified dishes with carbon films and coated with sericin than the PS control coated with sericin only.

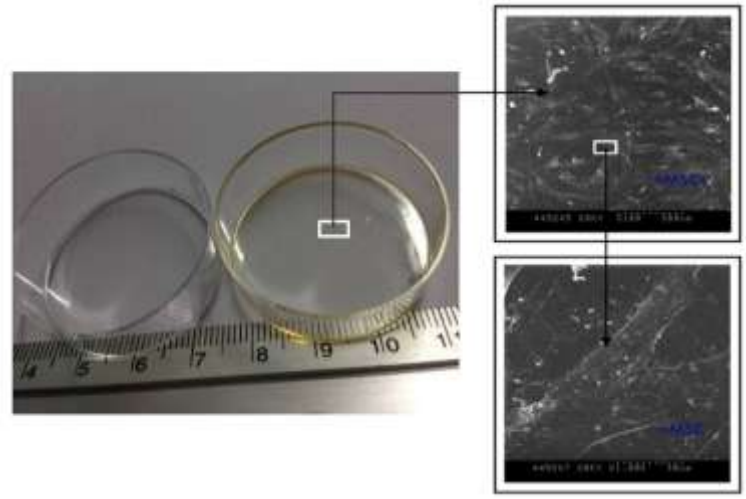

Fig. 9. The synthesized a-C based-films with sericin (right) can support cell proliferation.

2.2.2. A wound dressing surface modification method was developed by using PECVD of mixed ammonia (NH_3) and acetylene (C_2H_2). Plasma active species such as carbon and nitrogen radicals form film with active sites to create a covalent bond between Ag-SD compound and the cotton gauze surface. This novel method was targeted at controlling the delivery of the Ag-SD release while covering and protecting the wound, reducing the drug dosage and dosing times as well as keeping the wound dry by additional water repellant property.

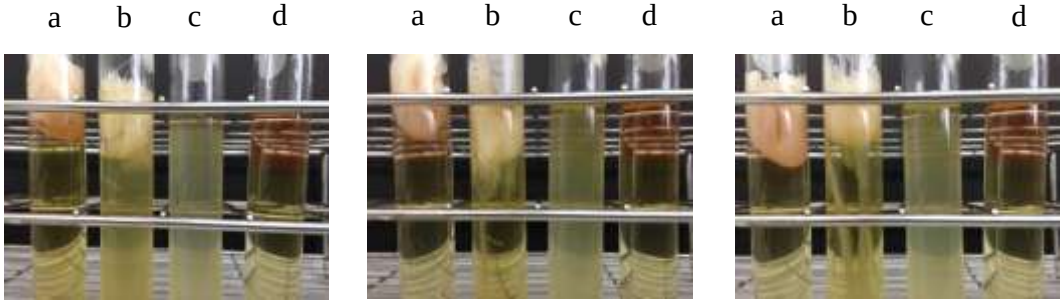

Fig. 10. The antimicrobial activity of modified gauze dressing (a) un-modify (b) control (c&d).

Fig. 10 shows the testing result on the effectiveness of the cotton wound dressing on antibacterial after exposure to *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. The plasma-treated gauze dressing plus Ag-SD exhibited clear appearance of the Fluid Thioglycollate Medium (FTM) as shown in tube a, indicating no bacterial growth, when compared with turbid appearance which indicated bacterial growth in gauze dressing standard and the control. This result demonstrates that plasma-treated gauze dressing performs well effectively to inhibit growth of tested bacterial strains which are commonly found on wound.

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