The influence of cross-linker concentrations on the properties of highly porous hyaluronic/gelatin sponges prepared by solvent casting/particulate leaching method for tissue engineering

INTRODUCTION:
Biomaterials that are composed of the extracellular matrix (ECM) are usually employed to improve cell attachment. Hyaluronic acid (HA) is a member of glycosaminoglycans (GAGs) that is present in the ECM of liver and has been found to actively influence the cellular adhesion, migration, and proliferation. To improve performance in HA-based scaffolds, there is a need to use a collagen (i.e. gelatin) which is soluble and resorbable in vivo [1]. In the present study, chemical cross-linking of gelatin based HA/gelatin scaffolds were studied with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) which acted as the cross-linker. The solvent casting and particulate leaching (SCPL) method [2] was also tested in this study. The SCPL approach was commonly used to allow the preparation of porous structures with regular porosity. However, in most instances, biomaterials that were composed of the ECM were prepared base on the studies using scaffolds of random pore geometry through lyophilization.

The hypothesis is that the porous structural distribution could affect the biological presentations of HA/gelatin composite scaffold for tissue engineering, such as cell adhesion and differentiation. The objective of this study is to test the effects of using different porous structures with regular porosity and different quantity of EDC on the physico-chemical properties of HA/gelatin composite scaffold.

METHODS:
Gelatin powder and sodium hyaluronate (HA) were mixed in double-distilled water. The pore size was controlled by using the modified method of a solvent casting and particulate leaching (SCPL) method [2]. Different salt weight-ratio (1, 2 and 3 g respect to S1, S2 and S3 groups) was stirred with the colloid of gelatin/HA/water about 1 mL and well mixed. N-(3-Dimethylamino propyl)-N’-ethylcarbodiimide hydrochloride (EDC) was used as the cross-linker. Different amounts of 1% EDC of 500 μL, 600 μL, 800 μL and 1000 μL were added in the gelatin/HA/water/salt colloids to initiate 5 min reaction before salt leaching process. The hyaluronan/gelatin sponges were then prepared by salt leaching and lyophilization processes. The strength, modulus, water absorption rates, bonding and morphologies of HA/gelatin sponges after cross-linking reactions were studied.

Statistical Analysis: ANOVA and Tukey-Kramer pairwise comparison were used to determine the statistical significance of the deviations in properties. In all cases the results were considered as statistically different with p < 0.05.

RESULTS:
The addition of larger size of salts led to decreased strength and modulus. (Fig.1a & 1b) The optimal addition quantity of EDC for higher strength was found to be 600 μL in tested specimens. Larger salt-leaching amount also led to higher water uptake, especially in 600 and 800 μL EDC groups. (Fig.1c) Tested samples showed no obvious degradation or dissolving phenomenon of external appearance in water. The different surfaces and internal morphologies of HA/gelatin sponges with or without water uptake were observed through OM (Fig.2a) and SEM analysis (Fig.2b). The higher interconnected porous were shown in the S3 groups that implied the group of s3 salt-leaching amount in SCPL process got more even porous-distribution ability. It could be seen from Fig.3 that obvious peak around 1060 and 1081 cm⁻¹ assigned to ester group was observed. However, the absorption bands corresponding to C–N–H stretching vibration were at about 1633, 1538 and 1447 cm⁻¹ where the absorption bands became more intense in the spectra of crosslinked HA/gelatin sponges. The clear intensities of the absorbance bands which could be assigned to the amide groups were different from HA or gelatin (Fig.3). This indicated the formation of the new crosslinking bonds (–CONH–) [3–4].

DISCUSSION:
The porous diameter of pores after water uptake was larger in the samples when compared to dried samples. This phenomenon was due to the balance of the swell expansion and surface tension effects in the sponges after water uptake. The measured porosities of the hyaluronic/gelatin sponges through image software were reduced after SCPL process and only the S3 group had the porosity larger than 70%. The highly interconnective pores of sponges were observed in the S3 group. This cross-linking reaction implied that the cross-linking bonds were mainly ester bonds which could easily be damaged in aqueous solutions and some –NH–CO– bonds were initially formed by EDC due to the cross-linking reactions between –COOH and –NH₂ groups [5].

The cross-linker EDC had a critical role in affecting the physiochemical properties of HA/gelatin sponges. The salt amounts in SCPL process had large effect on the connective porous distributions and water uptake ability. Based on the experimental results, it had suggested that the HA/gelatin sponge prepared using the 600μL EDC as the initial cross-linker amount and 3g salt leaching amount per samples had better results of improving the physiochemical properties of hyaluronan/gelatin sponges in vitro testing.

REFERENCES:

Figure 2. Morphologies through different salt-leaching amounts with 600 μL cross-linker EDC by OM (a) and SEM (b).

Figure 3. FTIR absorption bands of original chemicals and through different salt-leaching amounts after 600 μL EDC cross-linked.

Table 1. One-way ANOVA statistical analysis of scaffold Yield strength, modulus and water absorption abilities.

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