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Viscoelastic behavior of nano-hydroxyapatite reinforced poly(vinyl alcohol) gel biocomposites as an articular cartilage

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Abstract Nanohydroxyapatite reinforced poly(vinyl alcohol) gel (nano-HA/PVA gel) composites has been proposed as an articular cartilage repair biomaterial. In this paper, nano-HA/PVA gel composites were prepared by in situ synthesis nano-HA particles in PVA solution and accompanied with freeze/thaw method. The influence of nano-HA content, PVA concentration, test frequency and freeze/thaw cycle times on the viscoelastic behavior of nano-HA/PVA gel composites were evaluated using dynamic mechanical thermal analysis (DMTA). The results showed that both storage modulus and loss modulus firstly increased and then presented decreasing trend with the rise of nano-HA content. Their maximum values were obtained while nano-HA content was 6%. Furthermore, the G' and G'' of the composites improve with the increase of PVA concentrations and freeze/thaw cycle times. This effect was more distinct at low freeze/thaw cycles. The phase angle (tan δ) of the pure PVA gel is larger than that of the nano-HA/PVA composites at the test frequency spectra, but all the phase angle values of the tested composites were close to that of nature bone.

1 Introduction

For various daily activities in human life, tens of millions of people suffer from joint pain caused by the deterioration of articular cartilage, making it a prevalent cause of disability and deteriorating the daily life. Adult articular cartilage has a limited capacity to repair damage resulting from injury or disease. Replacement of articular cartilage is an effective surgical treatment to relieve pain and to restore the locomotion function for patients with damaged joints [1]. Recently, there have been many different approaches to restore tissue composition, structure, and function, including the development of engineered cartilage for potential implantation [2, 3].

It is found that poly (vinyl alcohol) (PVA) hydrogel is an excellent artificial articular cartilage biomaterial due to its biocompatibility and biotribological properties [4–7]. It possesses high porous structure and high content of free water, which is similar to that of natural articular cartilage. PVA hydrogel has increasingly attracted interest in application as a cartilage repairing material [8, 9]. However, the major problem of PVA hydrogel used in clinical application is the articular cartilage fixation method. Since PVA hydrogel itself does not adhere to tissue due to its non-bioactivity, long-term fixation of PVA hydrogel implant by suture is difficult.

Nano-hydroxyapatite (Nano-HA) has been applied widely in medical field as a bone repair material because of its excellent bioactive and biocompatibility properties [10, 11]. Therefore, composition of nano-HA and PVA to prepare nano-HA/PVA gel composites not only improves the mechanical properties of the composites but also endows its excellent bioactivities. It can form bone-bonding with nature tissue through osteo-conduction mechanism [12–14]. This is the effective method to improve the adhesion properties between implanted material and nature tissue. Our previous study results showed that the static mechanical properties of nano-HA/PVA gel composite is similar to that of articular cartilage [15]. However, there has been little evaluation on the

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dynamic mechanical properties of nano-HA/PVA gel biocomposites.

In this paper, nano-HA/PVA gel composites were prepared by in situ synthesis of nano-HA particles in PVA solution combining with freeze/thaw method. The influences of various factors on the viscoelastic properties were investigated.

2 Material and methods

2.1 Materials

PVA was purchased from Shanghai Chemical Co., Ltd, with the saponification degree of 99% and number-average polymerization degree of 1,750 ± 50. For the synthesis of hydroxyapatite, $Ca(OH)_2$ (analytical reagent, with purity of 95 wt%) was obtained from Shantou Xilong chemical factory of Gangdong and H_3PO_4(analytical reagent, with purity more than 85 wt%) was supplied by Siopharm chemical reagent Co., Ltd. All chemicals were used without any further purification.

2.2 Preparation of HA/PVA gel composites

First, a certain quantitative of $Ca(OH)_2$ with purity of 95 wt% was added into the 220 ml distilled water to prepare calcium hydroxide suspension. Second, PVA was introduced into the calcium hydroxide suspension. Third, The mixed solution of $Ca(OH)_2$ and PVA was stirred continuously at 90 °C for 1 h. Fourth, H_3PO_4 solution was added drop-by-drop to the blending solution of $Ca(OH)_2$ and PVA. The solution was maintained thoroughly stirring at 90 °C during the course of the reaction. The quantities of the reactants were selected to provide a Ca/P molar ratio, *R*, of 1.67.

After the reaction, the solution was maintained stirring at 90 °C for another 14 h. Subsequently, the solution was kept at 60 °C for 30 min to eliminate air bubbles. Then, the mixture was poured into stainless steel container. Finally, nano-HA/PVA gel composites were obtained by subjecting the mixed solution to several repeated freeze/thaw cycles. In each cycle, the sample was frozen at about –20 °C for 12 h, and then thawed at room temperature for 6 h.

2.3 Viscoelastic properties testing

The most suitable experimental technique, which has been applied to investigate the rheological properties of biopolymer gels, is small-deformation measurements used to probe visoelastic properties within the linear regime. In this experiment, the viscoelastic properties of the biocomposites were investigated using a dynamic mechanical thermal analysis instrument (DMTA Q800, USA) with shear mode. In the DMTA technique, a sinusoidal shear strain is applied and the resulting stress is measured. In controlled strain tests, the governing equation of applied strain, γ, is

$$\gamma = \gamma_0 \sin(\omega t) \tag{1}$$

where γ_0 is the applied strain amplitude and ω is the frequency of the strain wave.

$$\tau = \tau_0 \sin(\omega t + \delta) \tag{2}$$

where the phase angle, δ, is zero for an elastic solid and 90° for a Newtonian fluid. Hence, whenever the tested material is viscoelastic, the phase angle will lie between 0° and 90°. The phase angle, δ, can be calculated through the following equation:

$$\tan(\delta) = \frac{G''}{G'} \tag{3}$$

G', and G'', represent the elastic modulus and loss modulus, respectively.

In our study, flat rectangular samples with 25 mm parallel plates were used. In order to define the linear viscoelastic range of the composites, first a strain amplitude sweep at ambient condition was carried out to characterize the range of the strain amplitudes. The results showed that the range of strain amplitudes over which linear behavior was relatively broad (up to 15%). For the dynamic mechanical analysis with shear experiments, the strain amplitude of 3% was used. The specimens were tested under the strain control mode at 37 °C with frequencies ranging from 0.1 to 10 Hz, which corresponds to a physiological meaningful time scale. The results will be expressed in terms of G', G'' and tan δ as a function of frequency. G' gives information about the energy storage capability of the material as well as a measure of its stiffness, G'' informs about the energy dissipation capacity of the material in term of heat, and tan δ presents the information about the damping ability of the material.

3 Results and discussion

3.1 Effect of test frequency

Figure 1 shows the effect of frequency on the storage modulus of the gel composites with various contents of nano-HA. As shown in Fig. 1, G' values of all the composites samples increase with the rise of test frequency. The increase of G' with the rise of frequency implies that gel composites maintain a comparatively strong network structure [16]. The change behavior of the loss modulus

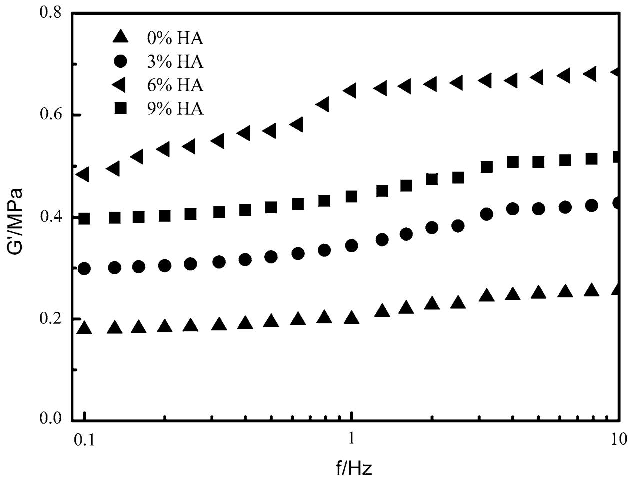

Fig. 1 Effect of frequency on the storage modulus of the gel composites with various contents of nano-HA

with the frequency shown in Fig. 2 is similar to that of *G*′ of the composites. The loss modulus displays weak dependence of frequency.

The viscoelastic behavior of the composites that the storage modulus, i.e. stiffness, increases with the rise of the frequency is similar to that of the natural articular cartilage. Such visoelastic characteristic of the composite is very important for its application as an articular cartilage biomaterial.

In the human activities, low frequency activities for the knee joint generally correspond to the normal activities such as walking and cantering, whereas the high frequency activities always correspond to the running and jumping activities. In low and high frequency conditions, the articular cartilage bears low and considerably high stress, respectively. In low stress conditions (low frequency activities), the stress which the articular cartilage endured can be uniformly distributed by enlarging the contact area of the articular cartilage due to its easy deformation at low stress level. While it is in the running and jumping conditions (high frequency activities), natural articular cartilage is expected to bear considerably high stress. The deformation of the articular cartilage has little increase and it presents excellent resistance to compressive stress at high loading force due to its increased stiffness. Such mechanical properties can insure the cartilage against damage caused by deformation under relatively high loading force.

While nano-HA/PVA gel composites are used as an articular cartilage repair material, low value of the storage modulus (stiffness) of the composites at low frequency endows the composites excellent deformation ability on low stress condition, whereas the composites have excellent resistance to external stress at high stress level due to its stiffness increasing with the rise of frequency. The response to different level external stress of nano-HA/PVA gel composites is very similar to that of articular cartilage.

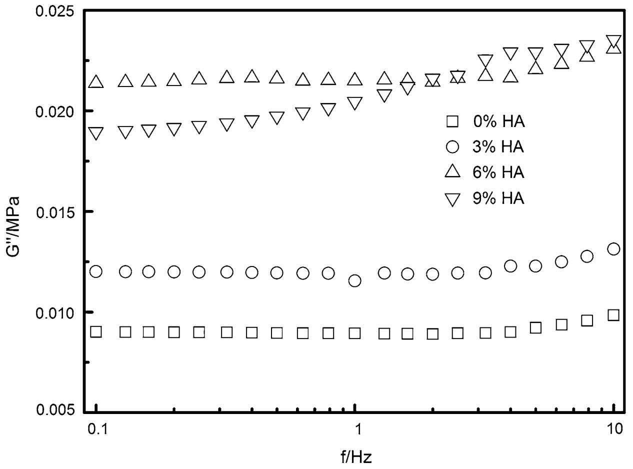

Fig. 2 Effect of frequency on the loss modulus of the gel composites with various contents of nano-HA

3.2 Effect of nano-HA content

It is well known that the incorporation of the nano-HA particles into the PVA matrix significantly alters the viscoelastic properties of such biomaterials. In these experiments, the ratio of water/PVA is kept constant (at 4) for a fair assessment of the contribution of the nano-HA to the elasticity of the biocomposites.

In order to simplify the comparison of the viscoelastic behavior of different composites, the indicated values of storage modulus, *G*′ and loss modulus, *G*″ at a single representative frequency (1 Hz) are plotted in Fig. 3 as a function of nano-HA content, although this approach does not characterize the complete viscoelastic behavior of the samples. It can be concluded from Fig. 3 that the *G*′ and *G*″

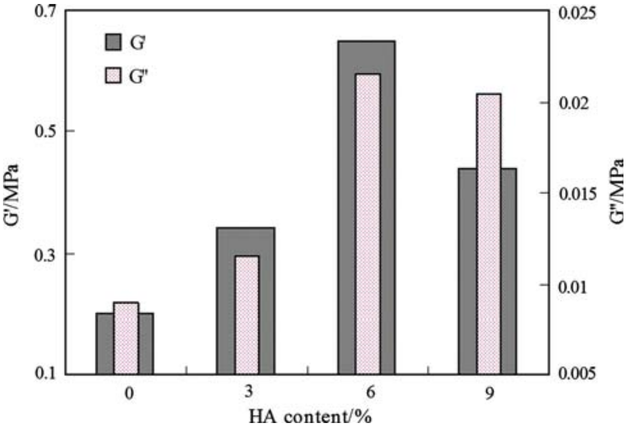

Fig. 3 Storage modulus, G′ and G″, at 1 Hz, as a function of the HA concentrations of the composites (PVA:H_2O = 1:4)

reach the maximum value while nano-HA content is 6%. For all the test composite samples, the results show that the G' values are approximately one order of magnitude higher than G'' values, showing that the elastic characteristic plays a principal role in the gel composites structure [17]. This result is also in agreement with the dynamical mechanical results of microbial alginate gels obtained by Moresi *et al.* [18].

It has been shown that the addition of inorganic nanoparticles such as silicon dioxide and hydroxyapatite to the polymer matrix can greatly affects the thermal, crystalline and rheological properties of the composites [19, 20]. The results of Xu *et al.* [21] showed that the calcium cation in hydroxyapatite and hydroxyl group in PVA molecule can form chemical bonding by the terms of [HO-]–Ca^{2+}–[-OH] linkage. The hydroxyl ions in the PVA matrix act as an especially active site for the complexation of the calcium ions in the nano-HA. These complexes would further interact with phosphate ions. Thus, the interfacial bonding strength between nano-particle and polymer matrix is improved. Furthermore, the addition of nano-HA into PVA matrix makes the composites become stiffer due to the rigidity of nano-HA, especially at low HA content [19]. On the other hand, the nano-HA particles can easily agglomerate due to their high surface energy while the content of nano-HA exceed a certain percent. Ultimately, the agglomerated nano-HA particles deteriorate the mechanical properties of the composites.

In this experiment, it has been suggested that the calcium inorganic cation form cross-links between two hydroxyl groups of PVA molecules while nano-HA content is less than 6% and the nano-HA particles are more likely to aggregate in the PVA matrix while its content is larger than 6%. The increase in the G' of our samples at low nano-HA concentration could be taken as an indicator that these cross-links strengthen the network structure of PVA hydrogel, resulting in stronger gel networks with higher elastic or storage modulus. The decrease in the G' at high nano-HA concentrations could be attribute to the aggregation effect of nano-HA particles.

The ratio between the loss modulus and the storage modulus is described by the phase angle (tan δ). Fig. 4 shows the phase angle as a function of frequency for the samples with different nano-HA contents. The result indicates that the phase angle of all the samples decrease with the increase of frequency, especially at low frequency region. Furthermore, the phase angles of the nano-HA/PVA gel composites, except gel composites with 9% nano-HA content, are less than that of pure PVA hydrogel, showing a higher elastic behavior with the addition of nano-HA in the PVA gel matrix. In the nano-HA/PVA composites, the PVA hydrogel matrix can be seen as a three-dimensional network, where the intermolecular space is mostly intimately occupied by the nano-HA particle crystals. Thus the interaction between nano-HA and PVA matrix forms, such interactions may restrict the rotational/translational mobility within the PVA chains and further influence the viscoelastic properties of the organic phase. The maximum phase angle observed in pure PVA gel could be due to the lack of interactions between the organic and inorganic phases [22]. It can be concluded that nano-HA can be considered as the elastic component which enforces the rigidity of the composites compared with pure PVA gel.

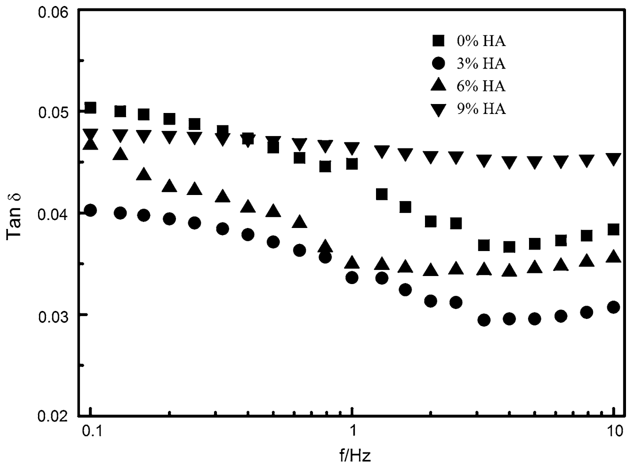

Fig. 4 Tan δ data as a function of frequency for samples with different HA contents (PVA:H_2O = 1:4)

The phase angle of PVA gel is higher than that of nano-HA/PVA gel composites, which suggests that the ability to absorb energy of PVA gel should be stronger than that of nanocomposites under an acute strain environment. The accumulation of absorbed energy will cause thermal degradation and further reduce the mechanical properties of materials [23]. Therefore, the thermal stability of nano-HA/PVA gel biocomposites with smaller phase angle is better than that of pure PVA gel. On the other hand, the decreased tan δ with the increase of frequency means that the difference value between the storage modulus and loss modulus increases and the stiffness of the composites becomes higher and higher. Just as discussed above, this damping property of the composites is beneficial to make the composites bear high stress and protect it against damage at high frequency level.

Buechner *et al.* [24] collected a series of results of tan δ of bone in a frequency range of 10^{-7}–10^{7} Hz. The results showed the values for human bone vary typically between 0.02 and 0.04. Such values may be helpful in terms of specifications to develop new materials to be used in orthopaedic applications. In our experiment, the frequency used in DMTA studies corresponds to the frequency of human physiological activities such as walk, jump and run. The results show that the phase angles of all the samples vary typically between 0.03 and 0.05, which depends on the frequency and nano-HA content. This result is in good agreement with the results of Buechner.

3.3 Effect of PVA concentration

Figure 5 shows the effect of PVA concentration on the G' and G'' in the test frequency spectra. It clearly indicates that G' values of the composites increase with the frequency and PVA concentration. Furthermore, G' values of the composites are much higher than G'' values in the test frequency spectra, showing that the elastic characteristic plays a principal role in the composites structure. The increase of G' of the composites with the increase of PVA concentration may be explained by the increase in both the number and average size of junction points. The number of the PVA molecules in unit volume increases rapidly and the movement ability of the polymer molecule chain is restricted due to the rise of PVA concentration.

The effect of PVA concentration on the phase angle in the test frequency spectra is shown in Fig. 6. The results display that the phase angle decreases with the rise of PVA concentration and also slightly decrease with the increase of test frequency, especially at low frequency region.

It was known that nano-HA/PVA gel composites consist of three parts: nano-HA, PVA molecule and water. Nano-HA possesses elastic properties and PVA molecular presents viscoelastic characteristics. As water is essentially viscous, the increase of water content in a viscoelastic medium increases tan δ. According to the results of Joao [25], assume nano-HA and PVA molecular are viscoelastic component, the complex modulus of their mixture is shown as follows:

$$G_1^* = G_1' + iG_1'' \tag{4}$$

Due to water is a pure viscous element, then

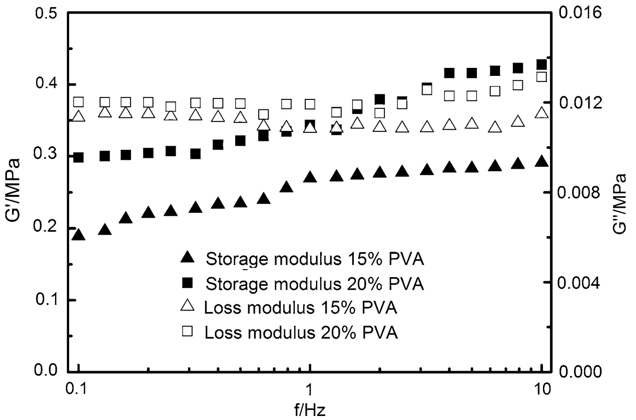

Fig. 5 Storage modulus, G′ (closed symbols), Loss modulus, G″ (open symbols) of the composites with different PVA concentration as a function of frequency (HA content: 3%)

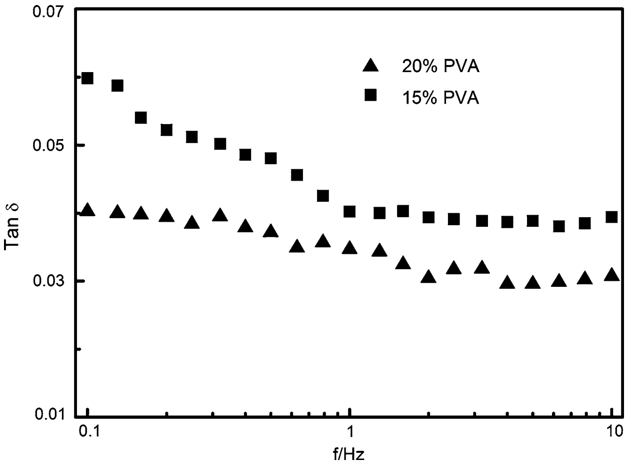

Fig. 6 The effect of PVA concentration on the phase angle (HA content:3%)

$$G_2^* = i\omega\eta \tag{5}$$

where ω and η are the angular frequency and viscosity, respectively, G_2^* is the complex modulus of the water. If the volume fraction of water is ϕ, according to the mixture theory of the composites, the complex modulus of the nano-HA/PVA gel composites is

$$G^* = (1-\phi)G_1^* + \phi G_2^* \tag{6}$$

Substitute (4) and (5) to (6) yields

$$G^* = (1-\phi)G_1' + i\,[(1-\phi)G_1'' + \phi\omega\eta] \tag{7}$$

According to formula (7)

$$\begin{aligned}\tan\delta &= G''\big/G' = [\phi\omega\eta + (1-\phi)G_1'']\big/[(1-\phi)G_1'] \\ &= \frac{\phi\omega\eta}{(1-\phi)G_1'} + \frac{G_1''}{G_1'}\end{aligned} \tag{8}$$

This quantity is higher than G_1''/G_1' and increases with the rise of ϕ. It should be noted that the decrease of PVA concentration correspond to the increase of water content in the composites. Therefore, it can be concluded that the damping in the composites should decrease with PVA content. This could explain why the studied samples with higher PVA content exhibits lower values of tan δ in the entire frequency scale analyzed.

3.4 Effect of cycle times

Figure 7 shows the storage modulus and loss modulus of the gel composite sample with 6% nano-HA subject to various freeze/thaw cycles at a single representative frequency (1 Hz). It can be concluded from Fig. 7 that both the values of G' and G'' increase with the number of freeze/thaw cycles. However, the increase rates of the storage

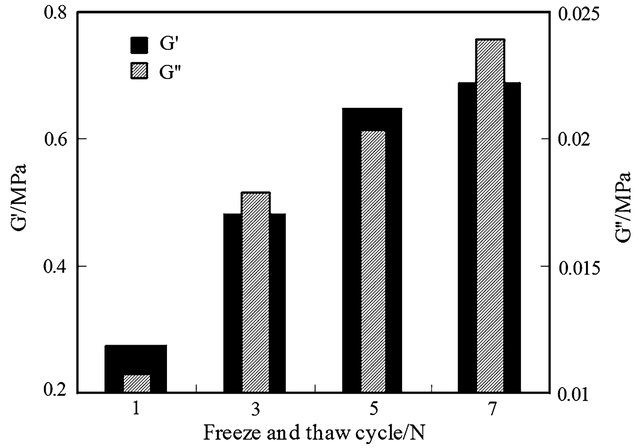

Fig. 7 Storage modulus, G′ and Loss modulus, G″, at 1 Hz, as a function of freeze and thaw cycle times (HA content: 6%; PVA:H_2O = 1:4)

modulus and loss modulus reduce with increased number of cycles. The most significant changes occur upon the 1 to 5 freeze/thaw cycle times. For example, the G' increases from 0.27 to 0.64 MPa and G'' increases from 0.011 to 0.021 MPa, almost increasing 1.37 and 1 times respectively, while the freeze/thaw cycle times rise from 1 to 5. On the other hand, while the freeze/thaw cycle times rise from 5 to 7, G' and G'' of the composites only increase 6% and 9% respectively.

PVA hydrogel is a semi-crystalline material where the microcrystalline and amorphous regions coexist. The improvement of the storage modulus of the composites with the rise of the freeze/thaw cycle times may attribute to the some factors: (i) Both the entanglement force of hydrogen bonding forms among the PVA polymer chains and the stability of the crystalline particles are enhanced with the increase of freeze/thaw cycle times [26]. (ii) The interstitial space of the network structure and the size of micro pores both reduce due to the more compact three-dimensional network of PVA hydrogel. (iii) The increase in the number of freeze/thaw cycles may lead to a successive increase in the number of crosslinks. These crosslinks would arise from the creation of free radicals due to the shearing produced while water crystals grow within the polymer solution in the freezing process [27]. (iv) A successive increase of the number of polymer chains which participate in the cooperative hydrogen bonds as the number of freeze/thaw cycles increases [28]. (v) For low freeze/thaw cycle times, an increase in the freeze/thaw cycle times or an increase in the polymer concentration induces an increase in the percentage of rigid protons, which is beneficial to the increase of elastic properties of the composites. After 5 or 6 freeze/thaw cycles, the percentage of rigid protons tends to a limiting value [29].

4 Conclusions

The viscoelastic behaviors of nano-HA/PVA gel composites were investigated. The following points can be summarized for the present investigation.

1. Both the storage modulus and loss modulus of the composites increase firstly and then decrease with the rise of nano-HA content in the PVA matrix. While nano-HA concentration is lower than 6%, the interaction between calcium and hydroxyl takes predominance effect on the viscoelastic behavior of the composites and results in the enhancement of its elastic properties. Contrarily, while nano-HA concentration is higher than 6%, nano-HA particles are more likely to aggregate, which deteriorates the mechanical properties of the composites.
2. Both the storage and loss modulus increase with the rise of PVA concentration because of the increase in crosslink junctions and more compact network structure in the composites. However, the phase angle (tan δ) decreases with the increase of PVA concentration.
3. Both G' and G'' increase with the number of freeze/thaw cycles, and the increase rate reduces at high numbers of freeze/thaw cycle times. The most significant changes occur upon the 1 to 5 freeze/thaw cycle times. The storage modulus of the nano-HA/PVA gel composites increase with the rise of test frequency.
4. The viscoelastic behavior of nano-HA/PVA composites is similar to that of natural articular cartilage and it is an excellent articular cartilage repair material considering the viscoelastic properties. For the nano-HA/PVA gel composites used as an articular cartilage, the optimum preparation process of nano-HA/PVA gel composites is 6% nano-HA in 20% PVA matrix repeated by 5 freeze/thaw cycle times.

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