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Fabrication of gelatin based composites containing bimetallic Ag-Au nanoparticles for biomedical applications

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Abstract: Recently, focus is directed on research which involves synthesis of nanoparticles using natural polymericmaterials that are eco friendly, inexpensive as well as biodegradable. In this study, bimetallic silver-gold nanoparticles (Ag-AuNPs) were synthesized usinggelatinextracted from chrome containing leather waste as a template.The nanoparticles were confirmed with UV-Vis and particle size analysis. The gelatin directed bimetallic nanoparticles were air driedto obtain as nanobiocomposites (NBCs). These NBCs were characterized via FTIR, DSC, TGA, XRDand SEM/EDX. Also, mechanical properties such as tensile strength and Young's modulus along with water absorption studies of the NBCs were conducted.The results indicate that the gelatin NBCs incorporated with bimetallicnanoparticles are biodegradable and possess the desired mechanical propertiesneededfor biomedical applications. Moreover, the *in vitro* results demonstrate the viability of fibroblast (NIH 3T3) cells with gelatin based NBCs.The gelatin NBCincorporated with Ag-Au NPs isbiocompatible and can be used as scaffolds which have potential for various biomedical applications.

Keywords: Gelatin, Bimetallic, Silver, Gold, Nanoparticles, Template, Nanobiocomposites.

I. INTRODUCTION

Gelatin, a derivative from the most abundant biopolymer collagen has been used in biomedical applications [1]. The physico-chemical and biological properties of gelatin render it as candidate suitable for fabricating composites and tissue engineered scaffolds [2-5]. Additionally, it is much cheaper compared to collagen and has reduced antigenicity which essentially makes it easily available and convenient to use as biomaterial [6-8]. It is well known that temperature above 37ºC results in gelatin polypeptide chains to unfold and functional groups such as $–NH_2$, –SH and –COOH present in these chains can serve as a template for synthesis of nanoparticles and in nano-sized devices fabrication [9].

Gold nanoparticles(AuNPs) possess unique optical properties and have found use in catalysis and in electronic devices [10]. Also, they have been used as therapeutic agents, sensory probes and in delivery of drugsfor biomedical applications [11,12].Similarly, silver nanoparticles also exhibit distinct optical activities that have found wide use in electronics, catalysis and in sensingbased applications [13,14]. Moreover, it displays antimicrobial activity against a broad spectrum of bacteria and fungi and thus finds use as a biocide and also in the preparation of bactericidal nanomaterials for wound dressings and surgical purposes [15-17].

The high surface energy and van der waal forces present in both silver and gold nanoparticle solutionsresultin their aggregation and therefore, capping of these nanoparticles is essential to avoid this phenomena [11]. Hence, there is a

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need and thus research towards syntheses of NPs using natural polymers that are eco friendly, cost effective as well as renewable materials have drawn attention.The side chain amino acids of the polymer such as gelatin interactmore effectively with thesenanoparticle surfaces. Thehigh surface area of these nanoparticles enables more non specific interactions with the side chain amino acids of the polymer namelygelatin. Although, limited research has been carried outto understand the interaction between gelatin and noble metal such as silver and gold nanoparticles, their use especially in biomedical applicationsis not well studied [9].

In the present investigation, gelatin derived from collagen extracted from chrome containing leather waste (CCLW) has been used as a template to synthesize Ag, Au and bimetallic Ag-Au nanoparticles. Gelatin acts as a reducing agent and also performs the role of a stabilizer in the nanoparticle synthesis. The air dried nanobiocomposites of gelatin directed bimetallic nanoparticles showed increased thermal stability, a parameter useful for biomedical applications. Also, these scaffolds possessed desired properties such as increased tensile strength along with water absorption capacity suitable for tissue engineering applications. Additionally, the *in vitro*studieswere carried out to evaluate the biocompatibility of these gelatin based scaffolds.

II. MATERIALS AND METHODS

a) Isolation of collagen from chrome containing leather waste (CCLW)

Collagen was extracted from the chrome containing leather waste following the procedure reported by Mandal et al. [18]. The CCLW was dechromed using concentrated sulphuric acid.The dechromed sample was then treated with 0.1M Tris HCl (pH 8), 0.2M β- Mercaptoethanol, 0.05M EDTA for 3 days. The collagen fibrils were suspended in 0.05M acetic acid with a sample/solution ratio 1:30 (w/v) containing pepsin 1:10,000 (w/w) at 4°C for 24 h. The pepsin solubilized collagen was centrifuged at 10000 rpm (7155 g) for 1 h. The supernatant was further dissolved in 0.05 M acetic acid; subsequently dialyzed and the collagen solution was stored for further use and analyses.

b) Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were prepared using collagen extracted from CCLW. The aqueous collagen solution with different solid concentrations (100 mL) was heated to 80±3°C. Collagen when heated to 80±3°C denatures to gelatin which acts as reducing/stabilizing reagent. 2 mL of the $AgNO_3$ solution (0.4 wt%) was added rapidly at a stirring rate of 3000 rpm . A color change from pale yellow to white was observeddue to the complex formation between gelatin and Ag^+ ion [9]. The reaction was carried out under dark conditions and the contents were subjected to vigorous stirring for 10 h at 80±3°C to ensure the complete formation of gelatin capped silver nanoparticles.

c) Synthesis of Gold Nanoparticles (AuNPs)

The aqueous gelatin solution with different solid concentrations (100 mL) was heated to 80±3°C.2 mL of $HAuCl_4$ solution (0.4 wt%) was added rapidly under vigorous stirring. The contents were further stirred for 4 h at 80±3°C, which resulted in purplish red gelatin-AuNPs solution.

d) Synthesis of bimetallic nanoparticles (Ag–AuNPs)

Silver–gold nanoparticles (Ag–AuNPs) were prepared using gelatin as reducing/stabilizing reagent. The aqueous gelatin solution (20 mL) was heated to 80±3°C. 10 mL of 0.01 M $AgNO_3$ was added to gelatin solution and stirred at 3000 rpm. Subsequently,10 mL of 0.01M $HAuCl_4$ solution was added to the contents and stirring continued for 5 h. The generation of purple color indicates the formation of gelatin-Ag-AuNPs.

III. RESULTS AND DISCUSSIONS

The collagenextracted from the chrome leather waste was confirmed by SDS-PAGE.The SDS-PAGE results shown in Fig.1ademonstratea distinct β band in addition to characteristic α bands (α_1 and α_2), which were the unfolding polypeptide chains of collagen triple helix [19]. This confirms the triple helical nature of the collagen. Also, the

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circular dichroism (CD) spectra of both collagen and aqueous gelatin solution when heated at 80±3°C were carried out (See Fig. 1b).

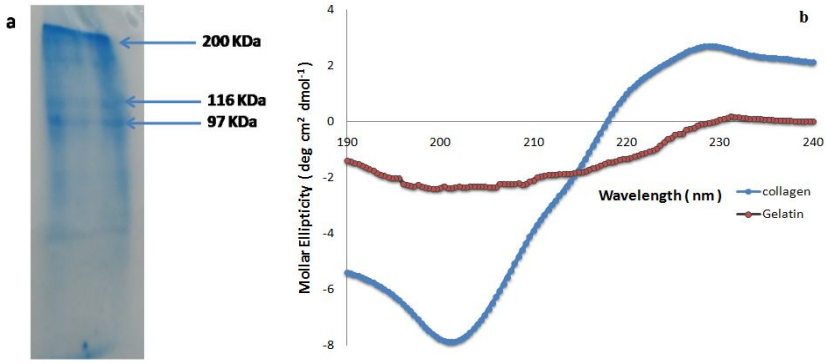

Fig. 1: a) SDS-PAGE of collagen extracted from CCLW; b) CD spectra of collagen and gelatin solution.

The collagen solution exhibited π-π* amide transitions at 198 nm and positive n-π* transition at 221 nm respectively and the intensity of the two bands, a measure of triple helical content of collagen molecules, was compared and found to match well with the CD pattern available in the literature. However, these characteristic bands were totally diminished when the aqueous collagen solution was heated at 80±3°C. This indicates the loss of triple helix of collagen leading to the formation of gelatin.

The synthesized nanoparticles were confirmed using UV-Vis spectroscopy as depicted in Fig.2. The gelatin capped silver nanoparticles showed the characteristic plasmon absorbance band at 415 nm. Similarly, the absorbance band at 556 nm was observed for gelatin assisted gold nanoparticles. In the case of bimetallic nanoparticles, the broad band at 485 nm intermediate to the pure Ag and Au NPs was noticed.

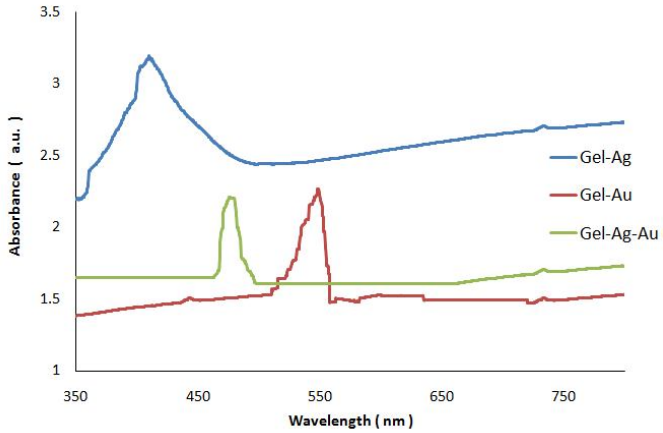

Fig. 2: UV-Vis spectra of gelatin assisted silver, gold and bimetallic nanoparticles.

Particle size analysis was used to determine the size of the synthesized particles. The gelatin capped silver and gold particles were in the range of 10-50 nm whereas the size of the gelatin coated bimetallic nanoparticles was found to be 30-80 nm respectively (See Fig.3).

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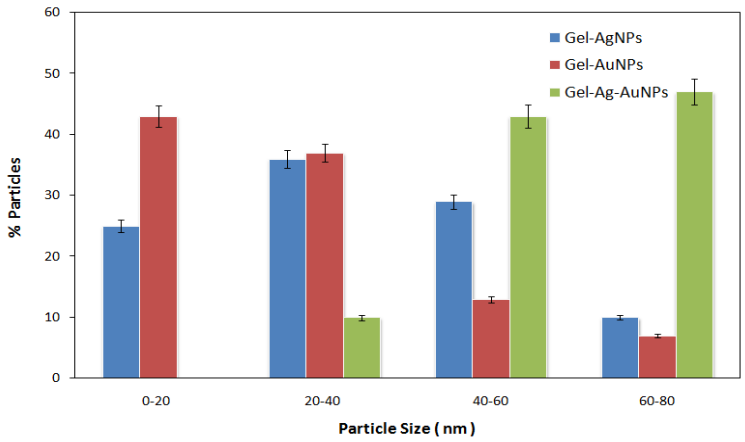

Fig. 3: Particle size distribution of gelatin assisted silver, gold and bimetallic nanoparticles.

Fourier Transform Infra red (FTIR) spectroscopy of nanobiocomposites (NBCs) containing gelatin capped nanoparticles were performed to study the interaction of silver, gold and the bimetallic (Ag-Au) nanoparticles with that of gelatin is demonstrated in Fig.4.The FTIR spectrum of gelatin alone show the characteristic amide I band at 1636 cm^{-1} due to the C=O stretching vibration of the amide group. Also, amide II band at 1548 cm^{-1} attributed to in plane N-H bending and C-H stretching of the amide group was noticed. Amide III band arising from the contributions of C-N stretching along with N-H in phase bending vibrations was detected at 1250 cm^{-1} which is associated with loss of triple helicity observed in gelatin molecules and the transformation of α-helical to random coil structure resulting from the collagen denaturation [20,21]. Moreover, the ratio (T_{1434}/T_{1240}) was less than 0.6 in all the NBC's which indicates lack of triple helical structure of gelatin [22].

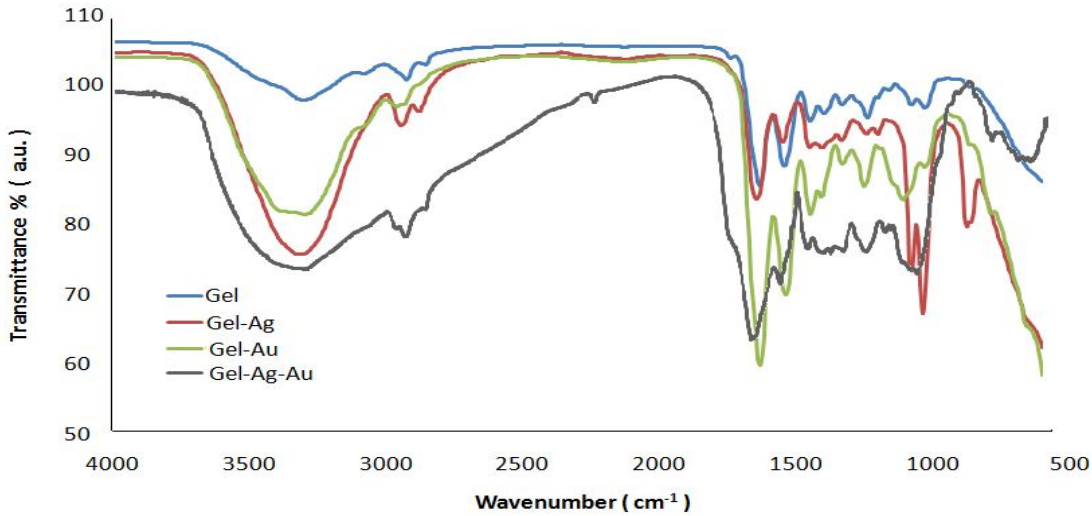

Fig. 4: FTIR spectra of gelatin (Gel), Gel-Ag, Gel-Au and Gel-Ag-Au NBCs respectively.

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The gelatin based NBC containing bimetallic Ag-Au NPs exhibited the maximum peak shift. Also, a peak at 1550 cm^{-1}due to the NH_3^+ group in the gelatin was noted. The adsorption of Ag, Au and the bimetallic Ag-Au NPs on the surface of gelatin in the NBCs resulted in the peak shift to 1560, 1558 and 1555 cm^{-1} respectively. This indicates the interaction of synthesized NPs with that of gelatin. Also, the peaks noticed at 1087 and 1041 cm^{-1} due to vibrations of the C-C bonds of the gelatin is red shifted with the incorporation of the nanoparticles in the gelatin matrix. The maximum peak shift is observed in gelatin NBC containing bimetallic Ag-Au NPs.

The DSC investigations showed three endothermic peaks at 85, 152 and 251 °C were observed for gelatin NBC (see Fig. 5).

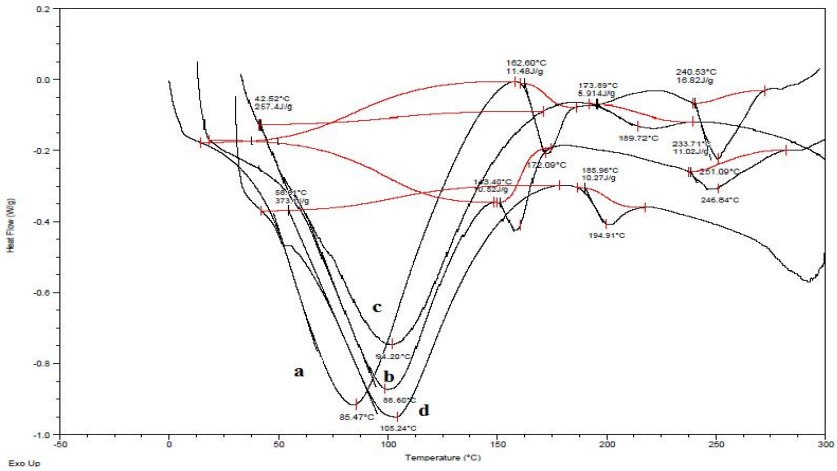

Fig. 5: DSC of a) Gel, b) Gel-Ag c) Gel-Au and d) Gel-Ag-Au NBCs respectively.

An increase in Tg values were noted in nanoparticle incorporated NBCs (Gel-Au, Gel-Ag) while the maximum values of 105, 195 and 285 °C were noted for Gel-Ag-Au NBC. The extra stability is most probably due to crosslinking of the bimetallic nanoparticles with that of the gelatin fibers. Also, it was observed from the thermogravimetric analysis (TGA) that the % of weight loss decreased with the addition of nanoparticles in the gelatin matrix. The weight losses of the gelatin based scaffolds at different temperature range are depicted in table 1.

Table 1: The mass losses for NBCs of gelatin (Gel), gelatin containing silver (Gel-Ag) and gold (Gel-Au) along with the bimetallic nanoparticles (Gel-Ag-Au) in different regions of temperature attained from thermogravimetric analysis.

Temperature range	**% mass loss of NBCs**			
	Gel	**Gel-Ag**	**Gel-Au**	**Gel-Ag-Au**
30-100 °C	10.7	9.8	9.2	8.7
100-200 °C	16.5	16.2	15.0	14.8
200-400 °C	66.8	65	62.7	61
800 °C (residue)	84.8	82.6	80.3	78.2

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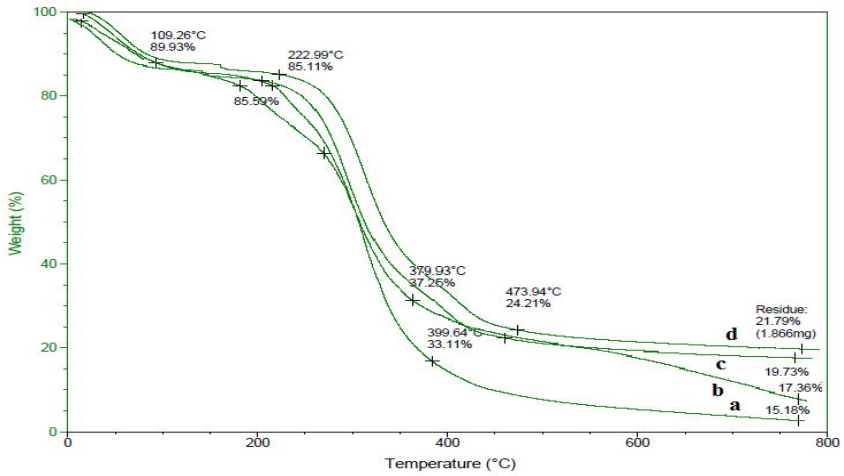

Fig. 6: TGA of a) Gel, b) Gel-Ag c) Gel-Au and d) Gel-Ag-Au NBCs respectively.

The gelatin NBC containing bimetallic nanoparticles (Gel-Ag-Au) showed the least weight loss (8.7%) compared to gelatin (10.7%) and gelatin scaffolds (10 and 9.2%) having individual silver and gold nanoparticles respectively (see Fig. 6). This is attributed to the added stability resulting from the crosslinking of gelatin with that of bimetallic nanoparticles (Ag-Au). The results are accordance with the findings of DSC.

The XRD pattern of the gelatin assisted Ag-AuNPs along with individual Ag and AuNPs are depicted in Fig. 7.

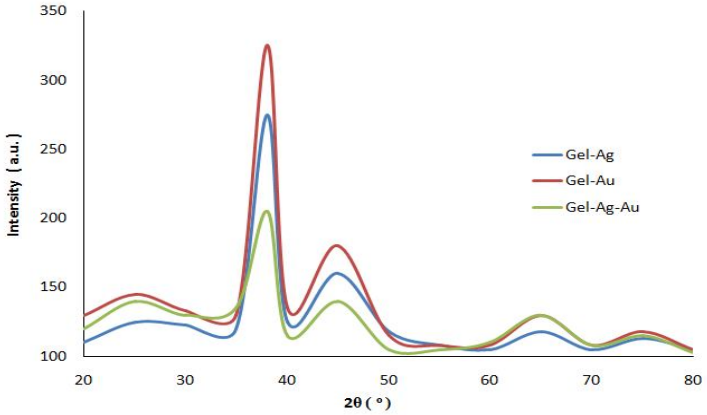

Fig. 7: XRD spectra of gelatin assisted silver, gold and bimetallic nanoparticles.

The broad peak at 23° indicates the amorphous nature of gelatin. Also, the characteristic peaks noted at 38° and 44.5° are due to Ag(110) and (200) faces. Moreover, the peak at 65° and 73 due to Ag (220) and Ag(311) were also noticed. Similarly, in the case of AuNPs, the peaks at 39° and 45° characteristic of Au(111) and Au (200) were also observed. The gelatin assisted Ag-Au NPs showed the characteristic peaks of both Ag and Au NPs. Thebroad peaks at 39° and 44.5° attributed to the Ag-Au (111) and Ag-Au (200) validate the formation of bimetallic NPs. The reduction in peak intensity is most probably due to the lower deviations of atoms in Ag-Au NPs from the ideal fcc lattice [9, 24].

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The surface morphology of the prepared scaffolds analyzed using SEM is depicted in Fig.8. The SEM images reveal the homogenous and uniform distribution of Ag and Au NPs in the gelatin scaffolds. Similarly, uniform distribution with very little aggregation was observed when bimetallic (Ag-Au) NPs were incorporated onto gelatin matrix. This has resulted in smooth surface of the scaffolds which is essential and a pre requisite of a material to be used for wound dressing purposes. It is well known that higher concentrations of gelatin results in the aggregation of the nanoparticles [4]. Therefore, in the present investigation, the bimetallic Ag-AuNPs and gelatin were mixed in the ratio 1:1 (v/v) to restrict the aggregation of the synthesized nanoparticles. The EDX spectra exhibit a strong signal of silver along with gold atoms which confirms the presence of both silver and gold nanoparticles in the gelatin scaffolds. Additional signals of C and O atoms observed in the gelatin scaffold are attributed to the carbon moieties and carboxyl groups present in the gelatin.

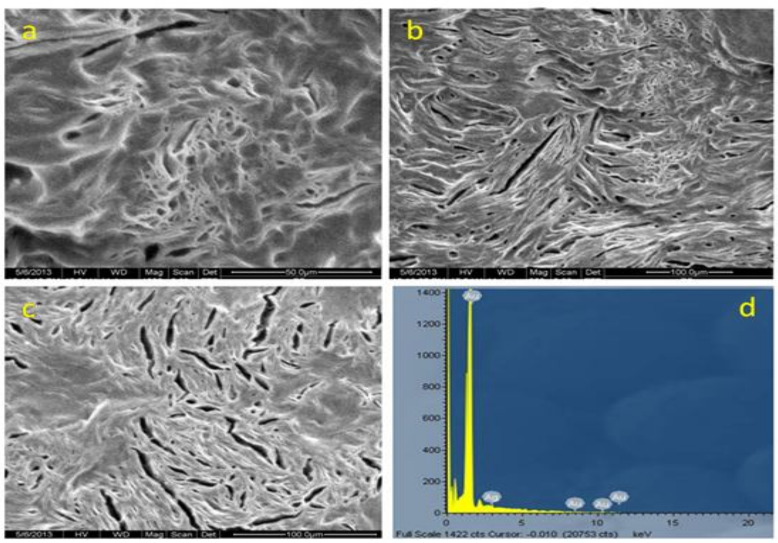

Fig. 8: SEM images of a) Gel-Ag b) Gel-Au c) Gel-Ag-Au and d) represents EDX spectrum of Gel-Ag-Au NBC.

The mechanical properties, tensile strength and percent elongation at break of the composites are illustrated in Table 2. Gelatin film demonstrated a tensile strength of 16.8±0.8 MPa. On the addition of AgNPs on to the gelatin composite (Gel-AgNPs), the tensile strength increases to 19.3±1.6 MPa. Similarly, incorporation of AuNPs in the gelatin based NBCdisplayed a value of 20.5±1.2 MPa. Further, increase in tensile strength (24.2±1.4MPa) was noticed for gelatin based NBCs containing Ag-AuNPs. This could be attributed to the incorporation of bimetallic nanoparticles that lead to entanglement ofgelatin chains and result in increased tensile strength of the NBC. Moreover, elongation at break (%) of the NBC's was also affected by the presence of nanoparticles. Significant decrease in elongation values observed is due to formation of cross links between the nanoparticles with that of gelatin chains.Thus, the developed NBC possess the desired mechanical properties and may find use as tissue engineered scaffolds for biomedical purposes.

The degradation of polymeric tissue engineered composites in humans is mainly based on its water uptake as hydrolysis is the prime mechanism that causes the polymer to degrade. The uptake of water content by the gelatin based NBCs was calculated using the formula [5,8]:

$$\text{Water uptake(\%)} = \frac{(W_h - W_d)}{W_d} \times 100 \ldots\ldots\ldots\ldots (2)$$

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where W_d and W_h are the initial dry weight and the hydrated weight of the NBC observed at different time periods, respectively.

Moreover, the porosities of the fabricated NBCs were obtained from the procedure followed by Shimizu et al. [25] and calculated using the following equation:

$$\text{Porosity (\%)} = \left[1 - \frac{(W_h - W_d)}{SV}\right] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where SV is scaffold volume; W_h and W_d are the hydrated and initial dry weight of the NBCs respectively.

Table 2. Mechanical properties of gelatin based nanobiocomposites (NBC's) obtained at 20ºC.

Composition	Tensile strength (in MPa)	Elongation at break (%)
Gelatin (Gel)	16.8±0.8	7.22±1.7
Gel-AgNPs	19.3±1.6	7.13±1.8
Gel-AuNPs	20.5±1.2	6.75±1.5
Gel-Au-AgNPs	24.2±1.4	6.1±1.3

The water absorption capacity (WAC)or the swelling ratio of the NBCshas significant importance as those NBCs with better WAC when applied on an open wound surface can easily absorb the exudates and allows the wound surface to remain dry and safe from air borne infections [26]. In the present investigation, all the NBCs exhibited high swelling ratio because of increase in water absorption and this increasedwith time with the maximum value attained at 24 h (See Fig.9). Also, it was noted that the presence of nanoparticles; both silver and gold in the gelatin NBC resulted in the lower swelling ratio (690±15%) due to decrease in water uptake. This is probably due to the fact that gelatin assisted template synthesis of nanoparticles resulted in their incorporation in the pores of the gelatin and thereby exhibited lower capacity to absorb water. The porosity of gelatin NBC was found to be 89% whereas NBCs when incorporated with nanoparticles resulted in decreased porosity (81 and 78% for Ag and AuNPs) and was least in the case of bimetallic Ag-Au NPs (71%). Thus, the gelatin NBC containing bimetallic Ag-Au nanoparticles displayed the least WAC as a porous NBC can take up and store more water compared to the non-porous ones. The result indicates that the developed NBCs are biodegradable and have the capacity to absorb water required for implantation applicationsespeciallyas materials for wound dressings.

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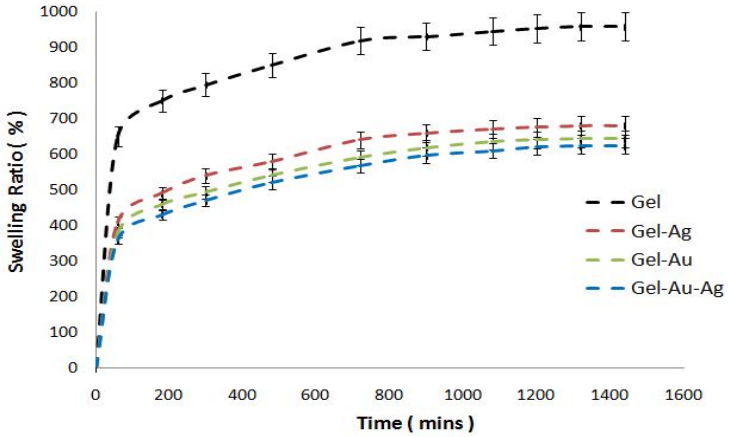

Fig. 9: demonstrates the swelling ratio of gelatin and gelatin based NBCs incorporated with silver, gold and their bimetallic nanoparticles.

The results of the cytotoxicity effects on fibroblast (NIH 3T3) cells cultured on the gelatin based NBCs containing Ag and Au NPs are illustrated in Fig. 10.The viabilities of NIH 3T3 cells shown as absorbance value at 540 nmforthe fabricated NBCs show excellent cytocompatibility with regard to tissue culture plate used as control.Addition of capped AgNPs on to gelatin NBC reduced the viability (85% and 91% at 24 and 48 h) of the fibroblasts.

However, the gelatin NBC containing AuNPs displayed increased cell viability (110% and 120% at 24 and 48 h) respectively. The incorporation of bimetallic Ag-Au NPs as expected also showed higher values (106% and 110% at 24 and 48 h) of fibroblast (NIH 3T3) cells on the gelatin NBC. This also indicates that the concentration of nanoparticles were within the safety limits and thus the bimetallic Ag-Au NPs incorporated gelatin NBC could be used as scaffold where the AgNPs provide antibacterial property while AuNPs enhances the biocompatibility required for tissue engineering applications.

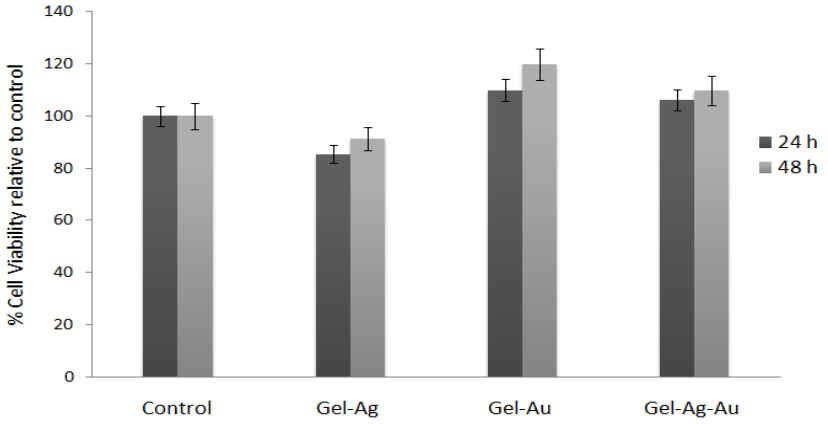

Fig. 10: Fibroblast (NIH 3T3) cell viability of gelatin based NBCs incorporated with silver, gold and their bimetallic nanoparticles relative to control at 24 and 48 h time period.

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This finding may be attributed to the high affinity of NIH3T3 cells to collagen and their migration around the NBCs. Moreover, it has been demonstrated that the presence of nanoparticles especially silver modulates the alignment of collagen and augment the fibroblast cell proliferation and its differentiation [27]. Here, in our case, the *in vitro* results suggest that the bimetallic(Ag-Au) nanoparticles impregnated in the gelatinNBC perform this function more effectively and can be used as scaffolds which are vital for clinical wound healing applications.

IV. CONCLUSIONS

Gelatin obtained from collagen extracted from CCLW serves as a template for the synthesis of Ag, Au as well as bimetallic Ag-Au nanoparticles and this green approach can be utilized to develop value added NBCs. The gelatin based NBCcontaining bimetallic Ag-Au NPs is biodegradable and possess enhancedstability; desired mechanical properties in addition to excellent biocompatibility demonstrated by the *in vitro* studies that can be used as scaffolds for various potential biomedical applications. Further *in vivo* studies to ascertain the validity of these gelatin based NBCs as wound dressing materials are required and work is being carried out in this direction.

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REFERENCES

[1] Olsen, D., Yang, C., Bodo, M., Chang, R., Leigh, S., Baez, J., Carmichael, D., Perala, M., Hamalainen, E.R., Jarvinen, M., Polarek, J.,"Recombinant collagen and gelatin for drug delivery",Adv. Drug Deliv. Rev.Vol. 55, no.12, pp.1547, 2003.

[2]Wan, Y., Zuo, G., Liu, C., Li, X., He, F., Ren, K., Luo, H.,"Preparation and characterization of nano-platelet-like hydroxyapatite/gelatin nanocomposites", Polym. Adv. Technol.,Vol.22, no.12,pp. 2659–2664,2011.

[3] Lien, S.M., Ko, L.Y., Huang, T.J. "Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering",Acta Biomater., Vol. 5, pp. 670–679, 2009.

[4] Mao, J.S., Zhao, L.G., Yin, Y.J., Yao, K.D. "Structure and properties of bilayer chitosan–gelatin scaffolds", Biomaterials,Vol. 24, pp. 1067–1074, 2003.

[5] Nguyen, T., Lee, B., "Fabrication and characterization of cross-linked gelatin electrospun nanofibers", J. Biomed. Sci. Eng., Vol. 3, pp. 1117-1124, 2010.

[6] Tabata, Y., Ikada, Y., "Vascularization effect of basic fibroblast growth factor released from gelatin hydrogels with different biodegradabilities", Biomaterials, 20, pp. 2169–2175, 1999.

[7] Ratanavaraporn, J., Damrongsakkul, S., Sanchvanakit, N., Banaprasert, T., Kanokpanont, S.,"Comparison of gelatin and collagen scaffolds for fibroblast cell culture",Journal of Metals, Materials and Minerals,Vol.16, no.1, pp. 31-36, 2006.

[8] Taokaew, S., Seetabhawang, S., Siripong, P., Phisalaphong, M., "Biosynthesis and characterization of Nanocellulose-Gelatin films",Materials,Vol. 6, pp.782-794, 2013.

[9] Liu, Y., Liu, X., Wang, X.,"Biomimetic synthesis of gelatin polypeptide-assisted noble-metal nanoparticles and theirinteraction study", Nanoscale Res Lett.,Vol. 6, no. 22,pp. 1-11, 2011.

[10]Murphy, C.J., Sau, T.K., Gole, A.M., Christopher,J.,Orendorff, Gao, J., Gou, L., Hunyadi, S.E., Li,T.,"Anisotropic metal nanoparticles: Synthesis, assembly and optical applications",J. Phys. Chem. B, Vol. 109, no. 29, pp. 13857–13870, 2005.

[11] Tagar, Z.A., Sirajuddin, Memon, N., Kalhoro, M.S., Kalwar, N.H., Junejo, Y., Hassan, S.S.,"Facile synthesis, characterization and catalytic function of gelatin stabilized gold nanoparticles", Pak. J. Anal. Environ. Chem., Vol. 13, no. 1,pp.70-77, 2012.

[12] Huang, X., Jain, P.K., El-sayed, I.H., El-sayed, M.A.,"Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy", Nanomedicine, Vol. 2, no. 5, pp. 681-693, 2007.

[13] Nguyen, K.C., Seligy, V. L., Massarsky, A., Moon, T.W., Rippstein, P., Tan, J., Tayabali A.F."Comparison of toxicity of uncoated and coated silver nanoparticles",J. Phys.: Conf. Ser., 429,012025, 2013.

[14] Faraji, N., Yunus W.M.M., Kharazmi, A., Saion, E., Shahmiri, M., Tamchek, N., "Synthesis, characterization and nonlinear optical properties of silver/PVA nanocomposites", J.Europ. Opt. Soc. Rap. Public, Vol.7, pp. 12040, 2012.

[15] Ansari, M.A., Khan, H.M., Khan, A.A., Malik, A., Sultan, A., Shahid, M., Shujatullah, F., Azam, A., "Evaluation of antibacterial activity of silver nanoparticles against MSSA and MRSA on isolates from skin infections", Biology and Medicine,Vol. 3, no. 2, pp. 141-146, 2011.

[16] Mandal, A., Sekar, S., Chandrasekaran, N., Mukherjee, A., Sastry, T.P. "Poly(ethylene) glycol capped silver and magnetic nanoparticles: Synthesis, characterization and comparison of bactericidal and cytotoxic effects", Proc IMech E Part H Eng Med., Vol. 227, no. 11, pp. 1224-1236, 2013.

[17] Sekar, S., Manikandan, R., Mandal, A., Sankar, S., Sastry, T.P., "Synthesis and characterization of chemically modified collagen based nanobiocomposite containing silver nanorods incorporated with ciprofloxacin", IJIRSET, Vol. 3, no. 2, pp. 9618-9625, 2014.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 5, May 2014

[18] Mandal, A., Sekar, S., Knagavel, M., Chandrasekaran, N., Mukherjee, A., Sastry, T.P., "Collagen based magnetic nanobiocomposite as MRI contrast agent for cancer therapy", BBA Gen Subjects, Vol. 1830, no. 10, pp. 4628-4633, 2013.
[19] Laemmli, U.K., "Cleavage of structural proteins during the assembly of the head of bacteriophage T4",Nature, Vol. 227, pp. 680-685, 1970.
[20] Muyonga, J.H., Cole, C.G.B., Duodu, K.G., "Fourier Transform Infrared (FTIR) Spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult nile perch (*Lates niloticus*). Food Chem,Vol. 86,pp. 325-333, 2004.
[21] Nikoo M., Xu X., Benjakul S., Xu G., Ramirez-Suarez J.C., Ehsani A., Kasankala L.M., Duan X., Abbas S, "Characterization of gelatin from the skin of farmed Amur sturgeon *Acipenser schrenckii*",Int Aquat Res, Vol. 3, pp. 135-145, 2011.
[22] George, A.,Veis, A.,"FTIRS in water demonstrates that collagen monomers undergo a conformational transition prior to thermal self-assembly in vitro",Biochemistry, Vol. 30, pp. 2372–2377, 1991.
[23] Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y., Roytrakul, S., "Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*", Food Chem, Vol. 116, pp. 445-451, 2009.
[24] Frenkel, A.I., Machavariani, V.S., Rubshtein, A., Rosenberg, Y., Voronel, A., Stern, E.A., "Local structure of disordered Au-Cu and Au-Ag alloys", Phys Rev B,Vol. 62, no. 14, pp. 9364-9371, 2000.
[25] Shimizu, K., Ito, A., Honda, H., "Enhanced cell-seeding into 3D porous scaffolds by use ofmagnetite nanoparticles", J. Biomed Mater Res. B Appl Biomater, Vol. 77, pp. 265-272, 2005.
[26] Devi, M.P., Sekar, M., Chamundeswari, M., Moorthy, A., Krithiga, G., Murugan, N.S., Sastry, T.P. "A novel wound dressing material-fibrin–chitosan–sodium alginate composite sheet",BullMater Sci, Vol. 35, pp.1157-1163, 2012.
[27] Kwan, K.H.L., Liu, X., To, M.K.T., Yeung, K.W.K., Ho, C., Wong, K.K.Y., "Modulation of collagen alignment by silver nanoparticles results in better mechanical properties in wound healing",Nanomedicine: NBM, Vol. 7, pp. 497-504, 2011.