Biocompatibility and Antioxidant Activity of a Novel Carrageenan Based Injectable Hydrogel Scaffold Incorporated with Cissus Quadrangularis for Facilitating Dentin-Pulp Complex Regeneration – An in vitro Study

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Abstract

Background: Over the past years, polysaccharide based scaffolds has been the most promising material for tissue engineering. In the present study, carrageenan, an injectable scaffold has been used owing to its advantage and superior property. *Cissus quadrangularis*, a natural agent was incorporated into the carrageenan scaffold which probably has the potential to make a favourable micro-environment for the dentin-pulp regeneration. Therefore, the present study aimed to assess the antioxidant activity and biocompatibility of the material.

Methods: The present *in vitro* study comprised of four study group each constituting a sample of 15 (n=15). The carrageenan hydrogel without addition of *Cissus quadrangularis* acted as control group (Group-I). Based on the concentration of aqueous extract of *Cissus quadrangularis* (10% w/v, 20% w/v and 30% w/v) in carrageenan hydrogel, respective study groups namely II, III and IV were considered. Antioxidant activity was assed using 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay and biocompatibility test was performed by using brine shrimp lethality assay. One-way ANOVA with the post hoc tukey test was performed using SPSS software v22.

Results: Significant difference (P<0.05) in the antioxidant activity was observed among the study groups with 20% w/v *Cissus quadrangularis* hydrogel (group III) reported the highest and control group showed the least antioxidant activity. A significant (P<0.01) drop in the antioxidant activity was observed in group IV when compared with group III. A significant (P<0.001) dose-dependent increase in biocompatibility was observed. Scanning electron microscopy analysis was performed to assess the surface morphology with different concentration of *cissus quadrangularis*, wherein group III showed even distribution throughout the hydrogel although the particles are close and densely arranged. Reduced antioxidant activity in group IV was probably due to clumping of the particles, thus reducing the active surface area.

Conclusion: Keeping the limitations of the in vitro study, it can be assumed that carrageenan based injectable hydrogel scaffold, incorporated with 20% w/v *Cissus quadrangularis* can provide favourable micro-environment as it is biocompatible and possess better antioxidant property. Hence, this study lays ground for future studies to explore this scaffold incorporated with *Cissus quadrangularis* for dentin pulp regeneration.

Background

During the embryonic development, the dentin and pulp originates from the dental papilla, thus forming a dentin-pulp complex which possess interrelated functions [1]. The later is essential for long term integrity of the tooth, however it is susceptible to various external stimuli [2] making it vulnerable to damage [3]. Clinically, the regenerative concepts have emphasized on treatment modalities such as pulp capping, pulpotomies and pulp revascularization with major emphasis on sensory nerve formation, blood vessel development and dentin formation [4]. Owing to its complex nature, the dental pulp is considered most

difficult tissue to regenerate [5]. Since, it's quite challenging to completely regenerate the dentin-pulp complex, it has laid pavement for various therapeutic regenerative protocols.

In recent days, there is an immense focus on recreating the lost architecture of human tissue or defective tissues; however, it's quite challenging [6]. Procedures such as harvesting a graft for regenerative purposes could lead to serious complications including pain, morbidity and higher risk of infection [6]. Hence, tissue engineering principles are employed for repair, regeneration and enhancing function of the defective tissues. Recent interest in tissue engineering is to assess the feasibility of regenerating an entire tooth or root which can be incorporated into the jaw bone [7]. The successful dentin-pulp regeneration depends on appropriate selection of stem cell type, scaffold material and bioactive factors [8].

Various tissue engineering approaches have been studied for dentin-pulp regeneration [9]. The stem cell-based tissue engineering concept emphasizes on combining the dental stem cells with a tissue-reparative microenvironment to promote dentin–pulp complex regeneration [3]. However, cell transplantation for dentin-pulp regeneration has numerous hurdles which make it difficult for the clinical translation [10]. Hence, the scaffold plays a major role in carrying incorporated stem cells to the site of therapeutic interest [11].

Various scaffolds containing polymeric materials, ceramics and bioactive glass have been used for dentin-pulp regeneration [12]. These scaffolds carry the bioactive molecules which can reside, stimulate and proliferate the resident tissue progenitor cells or stem cells [13]. Scaffold materials act as odontogenic mesenchymal stem cell (MSCs) extracellular matrix in the regeneration of dental pulp [14]. Any alteration in the composition, structure and mechanical properties, of scaffold will alter the biological properties of MSCs [15]. Currently, cell-free scaffolds are considered to be an alternative for dentin-pulp complex regeneration [16]. Among the available scaffolds, hydrogels are gaining more popularity in the field of tissue engineering [16].

The success of tissue regeneration depends on the material of choice. In this context, natural scaffold such as hydrogel claims to be a valid treatment option [17] as they carry a lower risk for cytotoxicity [16, 18]. They have unique three-dimensional polymeric network wherein water is the main liquid component. The hydrophilic nature of these hydrogels helps in retaining the higher fluid content and thus allowing the diffusion of nutrients through their structure [19]. They are biocompatible with adjustable mechanical properties and their cross-linking structure renders them less soluble despite of high water concentration [20]. They have a gelatinous structure, which provides an essential cell support, along with their ability to get loaded with various drugs, making them a good drug delivery system [12, 19, 21]. In the dentin-pulp regeneration process, the hydrogels being biodegradable allow the release of bioactive molecules influencing the surrounding environment [22, 23]. In addition to being a carrier for the cells or bioactive molecules, they are mainly applicable as a space-filling material for dentin-pulp regeneration [24]. Among various hydrogel formulations, [25] polysaccharide-based hydrogels have shown promising results in tissue engineering [26]. Being thixotropic in nature, they can be injected into the targeted space without

altering their physical, mechanical or biological properties [26]. Previous studies have shown the usage of hydrogels in both in vitro and *in vivo* settings for the pulp tissue cells regeneration [5, 27].

Tissue engineering is promising if stem cells are combined in injectable hydrogels. Their therapeutic success is primarily attributed to their ability of being injected inside the tooth and getting successfully adapted to the contours of the pulp chamber [16]. Carrageenan based hydrogel formulation is one such naturally occurring sulphate polysaccharide-based formulation having versatile properties [28]. Carrageenan is a natural compound obtained from red seaweed, which is a marine red algae [29]. Structurally, carrageenan has resemblance to the glycosaminoglycans which forms the extracellular matrix (ECM) of tissue, hence in physiological conditions, injecting this scaffold in the tissue defect will offer added benefits [30]. Although, it has not been explored much in dentin-pulp regeneration, but studies have found that carrageenan based extract is useful in the controlled delivery of drugs [31], bone tissue engineering purposes [32] as well as in wound healing [33].

Promising results have been documented with plant-derived bioactive compounds and its secondary metabolites in regenerative and therapeutic tissue engineering applications [34] including dentin-pulp regeneration [35]. *Cissus quadrangularis* is a vitaceae plant that has been used as a medicinal herb in India and Africa for ages. In traditional medicine, it is used for its antibacterial, analgesic, anti-inflammatory and antioxidant properties. Along with this it is employed for the purpose of bone fracture healing and prevention of osteoporosis [36]. Additionally, Calcium (about 4% by weight) and phosphorus ions are abundant in the *Cissus quadrangularis* stem extract [37].

Previous studies focused on evaluating the hydrogels incorporated with various natural compounds for dentin-pulp regeneration [16]. There are not of much research in the use of scaffold for dentin pulp regeneration, although previous literature evidence has shown hydrogel infused human dental pulp stem cells shown promising results in the regeneration. Evidence have shown collagen to enhance the dentin-pulp regeneration, [38] perhaps the use of scaffold in regeneration warrants more exploration. In dentin-pulp regeneration, as we are focused on achieving a favourable environment that could induce the dentinogenesis, currently studied extract, being anti-inflammatory along with being antioxidant, could favour the environment for biomineralization, ultimately leading to focused dentin-pulp regeneration. For a material to be used for dentin pulp regeneration, it is utmost important for it to be biocompatible and antioxidants are essential in preventing from oxidative stress thereby inhibiting from the damage caused by free radicals. Thus, the current study aimed at evaluating the activity of the *Cissus quadrangularis* incorporated in carrageenan based injectable hydrogel as a natural scaffold for facilitating the dentin-pulp regeneration. The present study is a preliminary one, which mainly focused on assessing the biocompatibility and antioxidant activity of a novel formulation of hydrogel in the *in vitro* models.

Methods

Study and Sample Characteristics

The present *in vitro* study was conducted after obtaining ethical approval from the institutional ethical committee (SRB/SDC/ENDO-2105/21/034). Depending on the con-centration of the *Cissus quadrangularis* aqueous extract, the current study had four study groups as mentioned below:

a. Group I: Carrageenan hydrogel (without any addition of *Cissus quadrangularis*, considered as control group(n = 15);
b. Group II: Carrageenan hydrogel with 10% w/v of *Cissus quadrangularis* aqueous extracts (n = 15)
c. Group III: Carrageenan hydrogel with 20% w/v of *Cissus quadrangularis* aqueous extracts (n = 15),
d. Group IV: Carrageenan hydrogel with 30% w/v of *Cissus quadrangularis* aqueous extracts (n = 15).

Sample Size Calculation

The sample size calculation was performed using G Power software. The effect size for the present pilot study was adjusted to 0.55. The alpha error was kept at 0.05 and the power of the study was 0.90. The minimal sample size of 15 was achieved per group, with a total sample size of 60.

Preparation of Hydrogel

Commercially available Carrageenan powder (Tokyo Chemical Indrustries (TCI), CASReg no: 11114-20-8, MeronTM, India), *Cissus quadrangularis* powdered extract (Annai Aravindh Herbals®,ISO 9001:2015 Certified SKU-AAH_PH_S_PROI, Chennai, India) and distilled water were used to prepare the hydrogel. A 100 mL of distilled water was heated at 60 ℃ for 30 min and 0.5 g of commercially available carrageenan powder was then dissolved in it by continuous stirring to make the carrageenan hydrogel. It served as the control agent in the study. A 10 g, 20 g, and 30 g of commercially available *Cissus quadrangularis* powdered extract (Annai Aravindh Herbals®, ISO 9001:2015 Certified SKU-AAH_PH_S_PROI, Chennai, India) were then dissolved in 100 mL of distilled water to produce 10% w/v, 20% w/v and 30% w/v aqueous extract of *Cissus quadrangularis* respectively. A 100 mL of this aqueous extract was then added to 0.5 g commercially available carrageenan powder and continuously stirred at 60 ℃ to form 10%, 20%, 30% w/v *Cissus quadrangularis* hydrogel [39]. The prepared hydrogel was then poured into standard moulds of dimension 6 x 2 x 2 mm3 and stored at 4 ℃. Before testing, the prepared hydrogel was sterilized using autoclaving for 15 min at 121℃ at 15 psi (Fig. 1A-E).

Testing for Antioxidant activity: DPPH Radical Scavenging Assay [40]

The prepared samples of the hydrogels were then subjected to antioxidant assay using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) model system. A 50 µL of control hydro-gel and different concentrations (10%, 20%, 30% w/v) of *Cissus quadrangularis* hydrogel were taken in test tubes. Later, 1 mL of a 0.1 mM methanolic solution of DPPH and 450 µL of 50 mM of Tris HCl buffer (pH 7.4) were added and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed depending on the absorbance at 517 nm. The butylatedhydroxytoluene (BHT) was employed as a control. The percentage of inhibition was determined from the following equation

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample x 100}}{\text{Absorbance of control}}$$

Testing for Biocompatibility: Brine shrimp lethality assay [41]

A 2 g of iodine-free salt was weighed and dissolved in 200 mL of distilled water. Fifteen, 6 well enzyme-linked immunoassay (ELISA) plates were taken and 10–12 mL of saline water was filled. Ten live nauplii were slowly added to each well. The wells were labelled as control, 10%, 20% and 30% according to the concentrations *Cissus quadrangularis* aqueous extract. A 50 µL of each concentration of hydrogel were added to each well as per their respective concentration and plates were incubated for 24 h. Later, the ELISA plates were observed and noted for the number of live nauplii present and calculated by using the following formula

$$\text{Percentage of live nauplii} = \frac{\text{Number of live Nauplii} \times 100}{\text{Number of live nauplii+Number of dead nauplii}}$$

Microstructure and Surface Morphology Analysis Using Scanning Electron Microscopy

The microstructure and surface morphology of the hydrogel samples containing different concentrations of *Cissus quadrangularis* aqueous extract was investigated using Scanning Electron Microscopy (SEM) analysis. The hydrogel samples were stored at – 50°C for 48 h and dried in a lyophilizer (VirtisBenchtop 4k freeze dryer). The cross-sectional surfaces of the sample in powder form were coated with a thin layer of platinum sputter and then SEM analysis was performed using field emission scanning electron microscopy (FESEM IT800).

Statistical Analysis

The gathered data was entered into the MS excel sheet. The normality of the data was assessed using the Shapiro-Wilk test. Data was presented in mean ± standard deviation and considering the parametric nature of data, One-way ANOVA with Post Hoc Tukey test was performed to derive inferential statistics. The data was analysed using IBM (IBM Corporation Business Analytics) SPSS software 23.0 version.

Results

Comparative analysis of Antioxidant Activity among Study Groups:

A statistical significant ($P < 0.001$) difference in the antioxidant activity was observed among the study groups with control (group I) and group III reported with the least and highest antioxidant activity (Fig. 2). On post-hoc evaluation, the group I exhibited significantly ($P < 0.001$) least antioxidant activity compared with other study groups. There was a non-significant (P 0.05) gradual increase in the antioxidant activity between group II and III. On the other hand, a significant ($P < 0.01$) drop in the activity was recorded with increased concentration of aqueous extract of *Cissus quadrangularis* hydrogel from 20% w/v (group III) to 30% w/v (group IV) (Table 1).

Table 1
Post-hoc evaluation of antioxidant activity among study groups

Pair-wise comparison groups		Mean difference	P Value	95% Confidence Interval	
				Lower Bound	**Upper Bound**
Group I	Group II	-3.867*	.000	-5.35	-2.39
	Group III	-4.400*	.000	-5.88	-2.92
	Group IV	-2.533*	.000	-4.01	-1.05
Group II	Group I	3.867*	.000	2.39	5.35
	Group III	− .533	.776	-2.01	.95
	Group IV	1.333	.092	− .15	2.81
Group III	Group I	4.400*	.000	2.92	5.88
	Group II	.533	.776	− .95	2.01
	Group IV	1.867*	.008€	.39	3.35
Group IV	Group I	2.533*	.000	1.05	4.01
	Group II	-1.333	.092	-2.81	.15
	Group III	-1.867*	.008€	-3.35	− .39

Note: p < 0.001; € P < 0.01

Comparative analysis of Biocompatibility among Study Groups

A statistical significant ($P < 0.001$) increase in the biocompatibility was observed among the study groups with group I and group IV reported with the least and highest level of biocompatibility (Fig. 3). On post-hoc evaluation, a significant ($P < 0.001$) increase in the biocompatibility was observed with each increment increase in concentration of aqueous extract of *Cissus quadrangularis* hydrogel (Table 2).

Table 2
Post-hoc evaluation of biocompatibility among study groups

Pair-wise comparison groups		Mean difference	P Value	95% Confidence Interval	
				Lower Bound	**Upper Bound**
Group I	Group II	-7.333*	.000	-9.52	-5.15
	Group III	-19.067*	.000	-21.25	-16.88
	Group IV	-22.333*	.000	-24.52	-20.15
Group II	Group I	7.333*	.000	5.15	9.52
	Group III	-11.733*	.000	-13.92	-9.55
	Group IV	-15.000*	.000	-17.18	-12.82
Group III	Group I	19.067*	.000	16.88	21.25
	Group II	11.733*	.000	9.55	13.92
	Group IV	-3.267*	.001€	-5.45	-1.08
Group IV	Group I	22.333*	.000	20.15	24.52
	Group III	15.000*	.000	12.82	17.18
	Group IV	3.267*	.001€	1.08	5.45

Note: p < 0.001; € P < 0.01

Comparative analysis of Biocompatibility among Study Groups

The SEM analysis of the control group revealed a porous structure of the hydrogel. It is essential for the regeneration as it provides a matrix for the stem cells of the apical papilla to embed and differentiate further into odontoblasts to lay down the dentin. In group II (10% w/v aqueous extract of *Cissus*

quadrangularis hydrogel), an even distribution of *Cissus quadrangularis* was seen throughout the hydrogel, whereas in group III, in addition to even distribution *Cissus quadrangularis*, the particles were shown to be close and densely arranged. The group IV, showed an uneven distribution of *Cissus quadrangularis* particles with the particles being clumped together (Figs. 4 and 5).

Discussion

Authors in recent years, research is ongoing to determine the use of hydrogel as a scaffold for regenerative procedures. Various natural, synthetic and hybrid hydrogels are available but the use of natural agents deliver more promising results by exhibiting lower cytotoxic response [18]. The ideal requirement for a scaffold to enhance the regeneration is to be biocompatible, having a neutral pH and should not induce inflammatory response [42]. Furthermore, it should be able to promote bioactivity, namely cell adhesion, proliferation and migration [43]. Although many studies have focused on the use of scaffold in tissue engineering, reports are scarce on the use of injectable carrageenan hydrogel scaffold infused with *cissus quadrangularis* in dentin-pulp complex regeneration. Hence, as a preliminary study, we focused on to assess the biocompatibility and antioxidant property of *cissus quadrangularis* incorporated carrageenan based hydrogel scaffold. A patent under the World intellectual property organization, with international publication no - WO 2008/081233 A2, with the inventor/applicant AVESTHA, GENGRAINE TECHNOLOGIES PVT LTD states that the percentage yield of whole plant extracts with active ingredients was the highest in water which was about 17.31%. The rest of the solvents used for extraction were hexane, 80% ethanol and acetone whose yields were much lesser than that of water. In the current study, this was the rationale of using aqueous extarct of *Cissus quadrangularis* for the preparation of hydrogel and further tests conducted [44].

In the present study the rationale for selecting the carrageenan hydrogel lies in the fact that it enhances favourable results in tissue regeneration process. This hydrogel has superior mechanical properties that depend on its molecular weight, source, concentration, type and degree of cross linkage [45]. The molecular weight of the hydrogel has an influence on its degradation property. It has been found that as the molecular weight increases the degradation rate decreases [46]. Studies showed that the degradation rate was higher when a lower weight scaffold such as chitosan-based hydrogels was used [47]. Furthermore, the three dimensional structure of carrageenan has shown osteoblastic proliferation and adhesion [48]. Combination of carrageenan hydrogels with different delivery system have shown successful outcome [49]. The prerequisites for an injectable hydrogel include flowability under low pressure, rapid setting at the target site and preserving the appropriate integrity and strength [50]. Due to its emulsifying and thixotropic property, it could be used as an injectable scaffold in enhancing the dentin-pulp regeneration [51].

In the present study, we have used a carrageenan based hydrogel incorporated with various concentrations of *Cissus quadrangularis* extracts which is a bioactive compound. To the best of our

knowledge, the later has not been explored for its therapeutic potential in dentin-pulp regeneration; however, it potential for osteogenesis has been extensively studied. The osteogenesis potential of *Cissus quadrangularis* extract has been explored in various dental clinical situations such as periodontal bone regeneration [52], mandibular alveolar ridge distractions [53] and in maxillofacial [54] and mandibular fractures [55]. In particular, the *Cissus quadrangularis* extracts have been shown to contain calcium, along with other compounds [56] and thus probably have shown to regulate osteoblastic activity [57] by enhancing osteoblastogenesis, [57] mineralization [58] and eventually induces bone formation for faster bone healing [59]. Literature also showed that *Cissus quadrangularis* extract stimulated the mineralised nodules in dental pulp cells [60].

It's known that maxillofacial structures, including the bone and dentition, originate from the same embryologic origin from the neural crest and share similar compositions in terms of organic and inorganic components [35]. Although, bone and teeth share a considerable amount of similarities, their developmental and regenerative properties differ due to their constructional proportions and their material and cellular compositions [61]. The cells of both the tissues secrete an almost similar extracellular matrix, which is termed as predentin as osteoid respectively [62]. The mineralisation of both dentin and bone extracellular matrix seemed to be initiated by a similar mechanism by the aid of matrix vesicles, later involving the secretion of families of specialised matrix proteins [63]. There are various non-collagenous proteins such as bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP) and osteopontin (OPN) that are not tooth-specific and found in both bone and dentin. They are expressed at varying levels and play a role in similar mineralisation mechanisms. As a result of their shared evolutionary history, bone and dentin have many characteristics in common. It's not surprising that the cells secrete matrices, osteoblasts and odontoblasts which are closely related cell types [64]. Recent literature found the stage-specific and tissue expression of BSP [65], OPN [65] in reparative dentinogenesis. On the other hand, studies are showing the efficacy of *Cissus quadrangularis* extract on OPN, [66] bone morphogenetic protein (BMP) [67] and BSP [68] activation, which in turn gives us an idea on usage of this current material for dentin-pulp regeneration, as the material has immense potentiality for osteogenesis and mineralisation.

Another favourable property of *Cissus quadrangularisis* is its antibacterial property. In previous studies, in order to prevent the growth of residual endodontic bacteria, chitosan was used as a scaffold material [69]. *Cissus quadrangularis* has demonstrated antibacterial efficacy against *bacillus subtilis, pseudomonas aeruginosa, salmonella typhi, escherichia coli, proteus mirabilis, staphylococcus aureus* and *streptococcus pyogenes* [70]. Along with this it has been shown effective antibacterial effect against oral microorganisms such as *S. mutans and L. Acidophilus* [71].

In the present study, *cissus quadrangularis* and carrageenan hydrogels are combined to assess the antioxidant activity and biocompatibility. Till date there are no reports on the usage of combinations of these injectable hydrogels for assessing these properties in dentin-pulp regeneration.

For the assessment of the antioxidant activity, DPPH test was performed in the present study as it has been popularly used to test the antioxidant properties of the plant extracts. In the DPPH assay, the addition of the extract to a violet-colored DPPH solution reduces it to a yellow-colored product, diphenylpicryl hydrazine in a concentration-dependent manner. The convenience offered by the short duration of the assay, allow its wide applications to predict antioxidant activity [72]. Dhanasekaran S et al. (2020) had evaluated the antioxidant property of *cissus quadrangularis* with different concentrations ranging from 25 to 400 µg/mL in ethanolic and methanolic extracts. In this *in vitro* model, the free radical scavenging activity was more in methanolic extract compared to ethanolic extract in a dose-dependent manner [40]. In the present study, 10 and 20% w/v *cissus quadrangularis* showed a significant gradual increase in the antioxidant activity as compared to the control group, however a significant drop in the activity was observed with further increase in the concentration of the agent to 30% w/v. On SEM analysis it was observed that 10% and 20% w/v *Cissus quadrangularis* hydrogel showed an even distribution of the incorporated agent throughout the hydrogel but when the concentration is increased to 30% w/v, an uneven distribution with clumping of the *Cissus quadrangularis* particles was noted. This could be a probable reason for the reduced antioxidant activity seen with the increasing concentration.

Another crucial factor required in the micro-environment for successful tissue engineering is biocompatibility of the scaffold. To evaluate the same, carrageenan based hydrogel infused with *Cissus quadrangularis* was subjected to brine shrimp lethality assay. Current study showed a significant increase in the biocompatibility with the increasing concentration of the additive agent in the scaffold. *Cissus quadrangularis* extract, has been extensively explored for its antioxidant, anti-inflammatory and bone tissue regeneration in various *in vitro* studies (34). Previous reports have shown better cytocompatibility of *Cissus quadrangularis* when performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for bone tissue engineering [34]. In another study, least cytotoxicity of carrageenan hydrogels was seen when performed on L929 fibroblast cells [30]. In the present study, *Cissus quadrangularis* and carrageenan hydrogels are combined to assess the antioxidant activity and biocompatibility. Till date there are no reports on the usage of combinations of these injectable hydrogels in dentin-pulp regeneration.

Limitations and Future directions

This is the preliminary *in vitro* study to assess the antioxidant and biocompatible property of carrageenan injectable hydrogel infused with *Cissus quadrangularis* for den-tin-pulp regeneration. The interaction between *Cissus quadrangularis* extract and carrageenan need to be studied further. Whether the sulphated groups and slightly acidic nature of the carrageenan inhibit the bio-active compounds from *Cissus quadrangularis* is unknown. Further studies need to be done using to stem cells from the apical papilla (SCAP) cell line to evaluate the biocompatibility of the hydrogel prepared. Studies need to be performed using organic and polar solvents to see if more active ingredients are released which can be used to synthesise a hydrogel with better antioxidant potential.

Conclusions

Within the limitation of the study, it can be concluded that 20% w/v Cissus qua-drangularis infused carregeenan based injectable hydrogel showed enhanced antioxidant activity whereas when the concentration of Cissus quadrangularis was increased to 30% w/v, has shown diminished response. However, there was a significant increase in the biocompatibility in a dose-dependent manner.

Declarations

Ethics approval and consent to participate

The present *in vitro* study was conducted according to the guidelines of the Declaration of Helsinki and approved by the institutional Ethics Committee (SRB/SDC/ENDO-2105/21/034) of Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India. Informed consent was waived by the institutional Ethics Committee of Saveetha Institute of Medical and Technical Sciences (SIMATS) due to *in vitro* nature of the study.

Consent for publication

Due to the *in vitro* nature of the study, it is not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declare no potential conflicts of interest with respect to the authorship or publication of this manuscript.

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Authors' contributions

All authors contributed to this article. “Conceptualization, S.S., N.M.S., K.V.T., and M.K.A; methodology, S.S., N.M.S., R.E, and S.M.P.; formal analysis, S.S. and K.C.S.; investigation, S.S., N.M.S., K.V.T., K.J., R.E, S.M.P.; resources, N.M.S., K.V.T., K.J., R.E, S.M.P., K.C.S.; data curation, D.S., M.A.O., H.A.A., M.M., A.R.Q., N.Q., K.C.S.; writing—original draft preparation, S.S., N.M.S., D.S., K.V.T., K.J., K.C.S.; writing—review and editing, S.S., N.M.S., D.S., M.A.O., H.A.A., M.M., A.R.Q., N.Q., K.V.T., K.J., R.E, S.M.P., M.K.A, K.C.S.;

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Figures

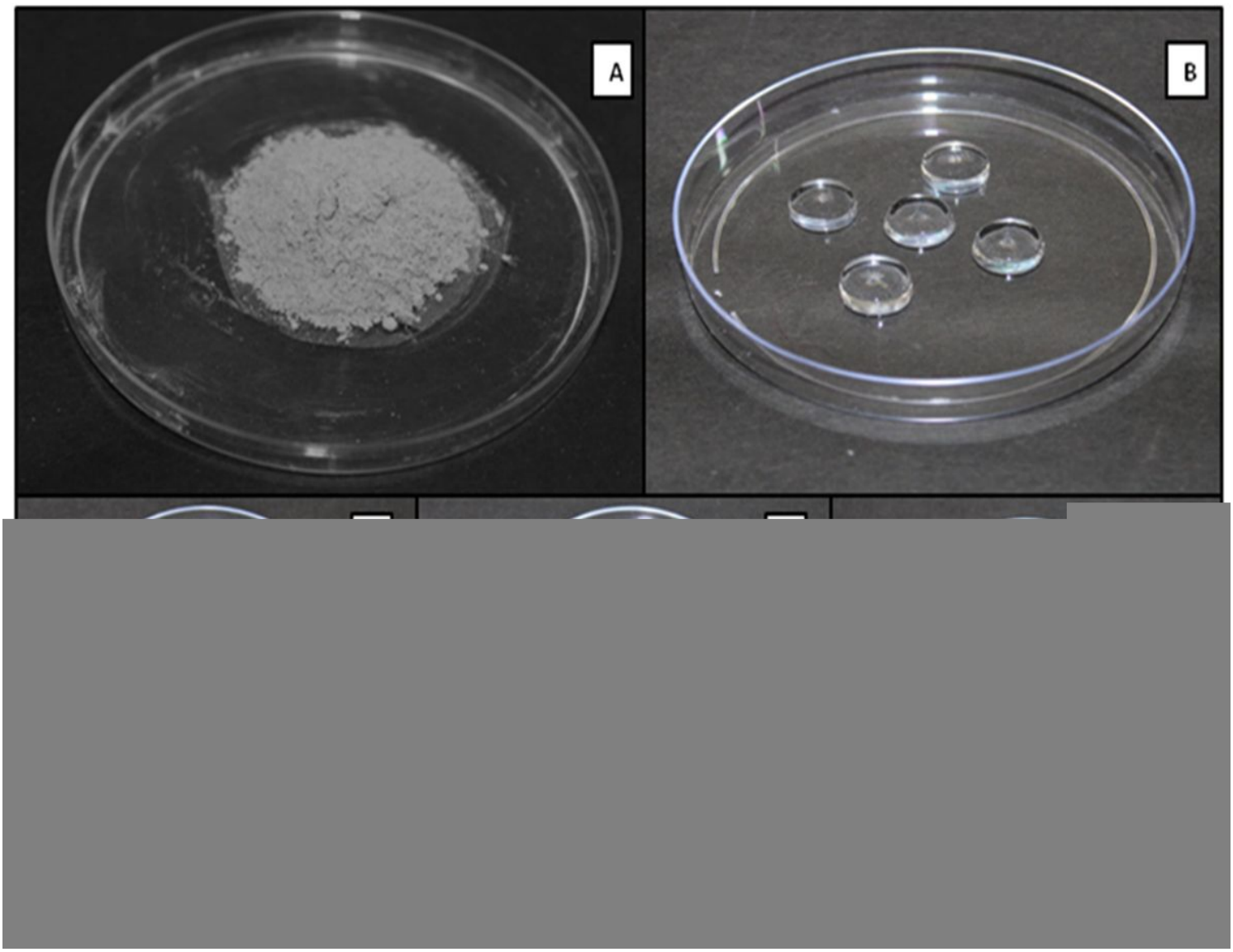

Figure 1

(A) Commercially available *Cissus quadrangularis* powder; (B) Carrageenan Hydrogel (Control); (C) 10% w/v *Cissus quadrangularis* hydrogel; (D) 20% w/v *Cissus quadrangularis* hydrogel; (E) 30% w/v *Cissus quadrangularis* hydrogel

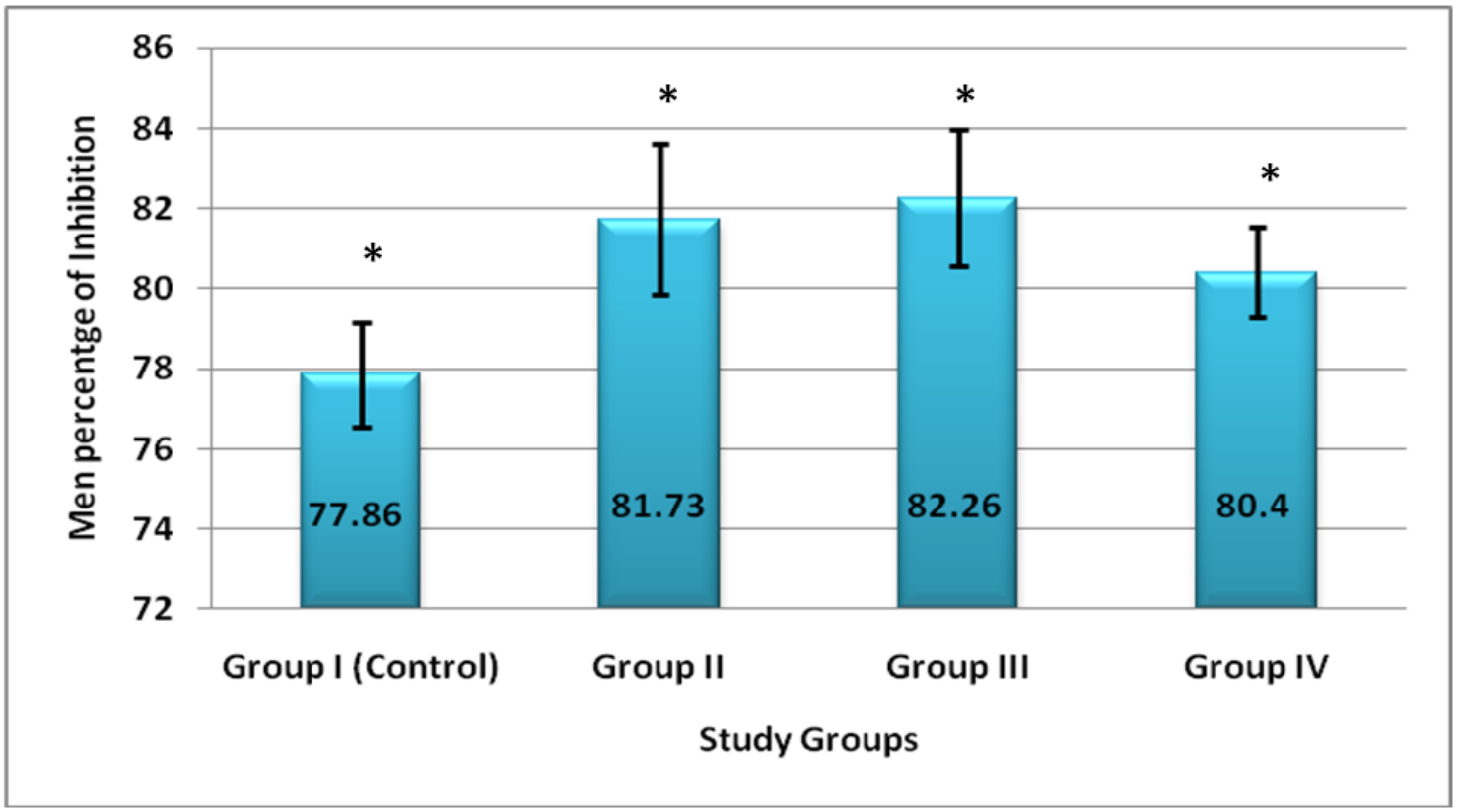

Figure 2

Comparative analysis of antioxidant activity among the study groups

Note: Group I: Carrageenan hydrogel (without any addition of *Cissus quadran-gularis*; Group II: Carrageenan hydrogel with 10% w/v of *Cissus quadrangularis* aqueous extracts; Group III: Carrageenan hydrogel with 20% w/v of *Cissus quadrangularis* aqueous extracts; Group IV: Carrageenan hydrogel with 30% w/v of *Cissus quadrangularis* aqueous extracts; *statistically significant.

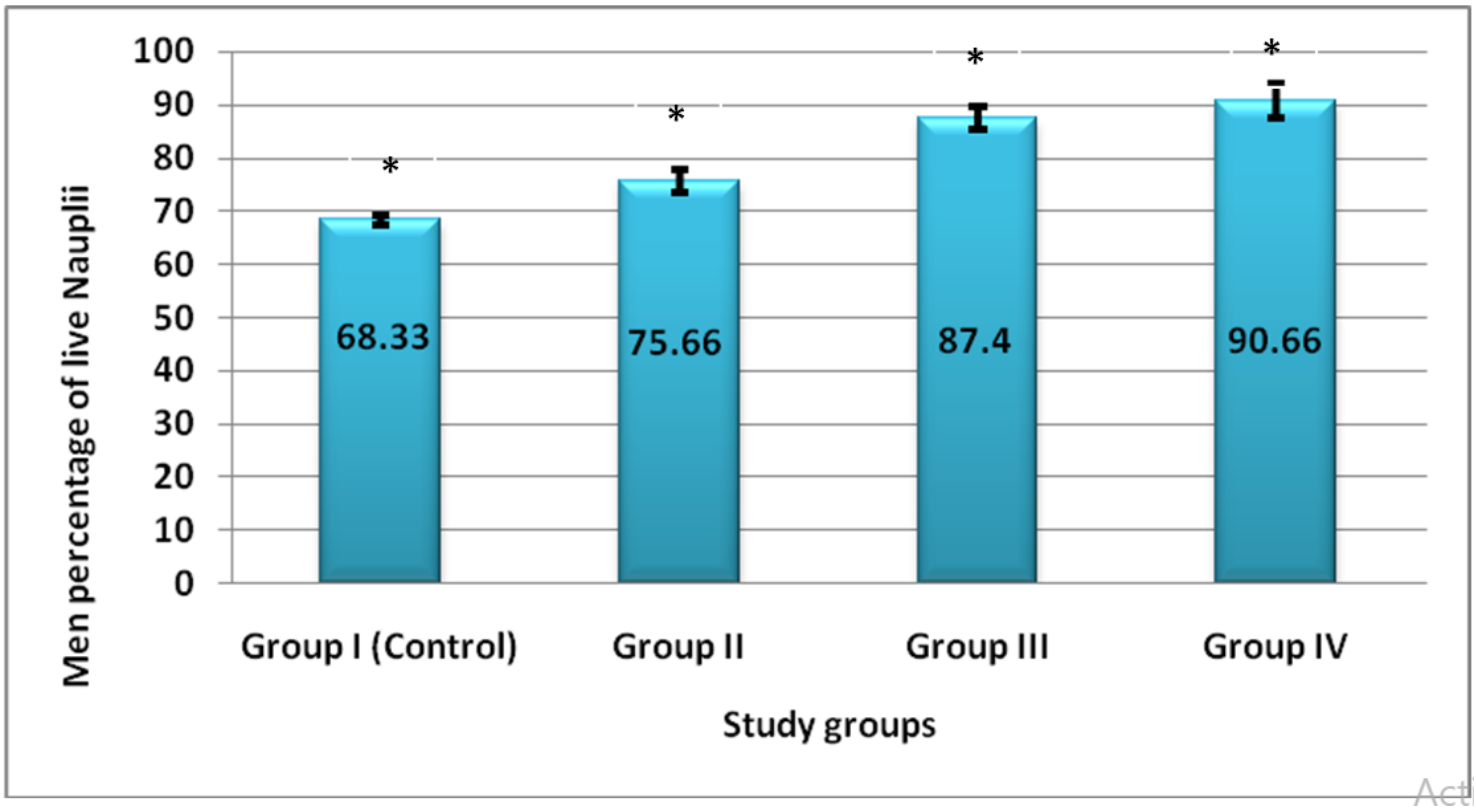

Figure 3

Comparative analysis of biocompatibility among the study groups

Note: Group I: Carrageenan hydrogel (without any addition of *Cissus quadrangularis;* Group II: Carrageenan hydrogel with 10% w/v of *Cissus quadrangularis* aqueous extracts; Group III: Carrageenan hydrogel with 20% w/v of *Cissus quadrangularis* aqueous extracts; Group IV: Carrageenan hydrogel with 30% w/v of *Cissus quadrangularis* aqueous extracts; *****statistically significant.

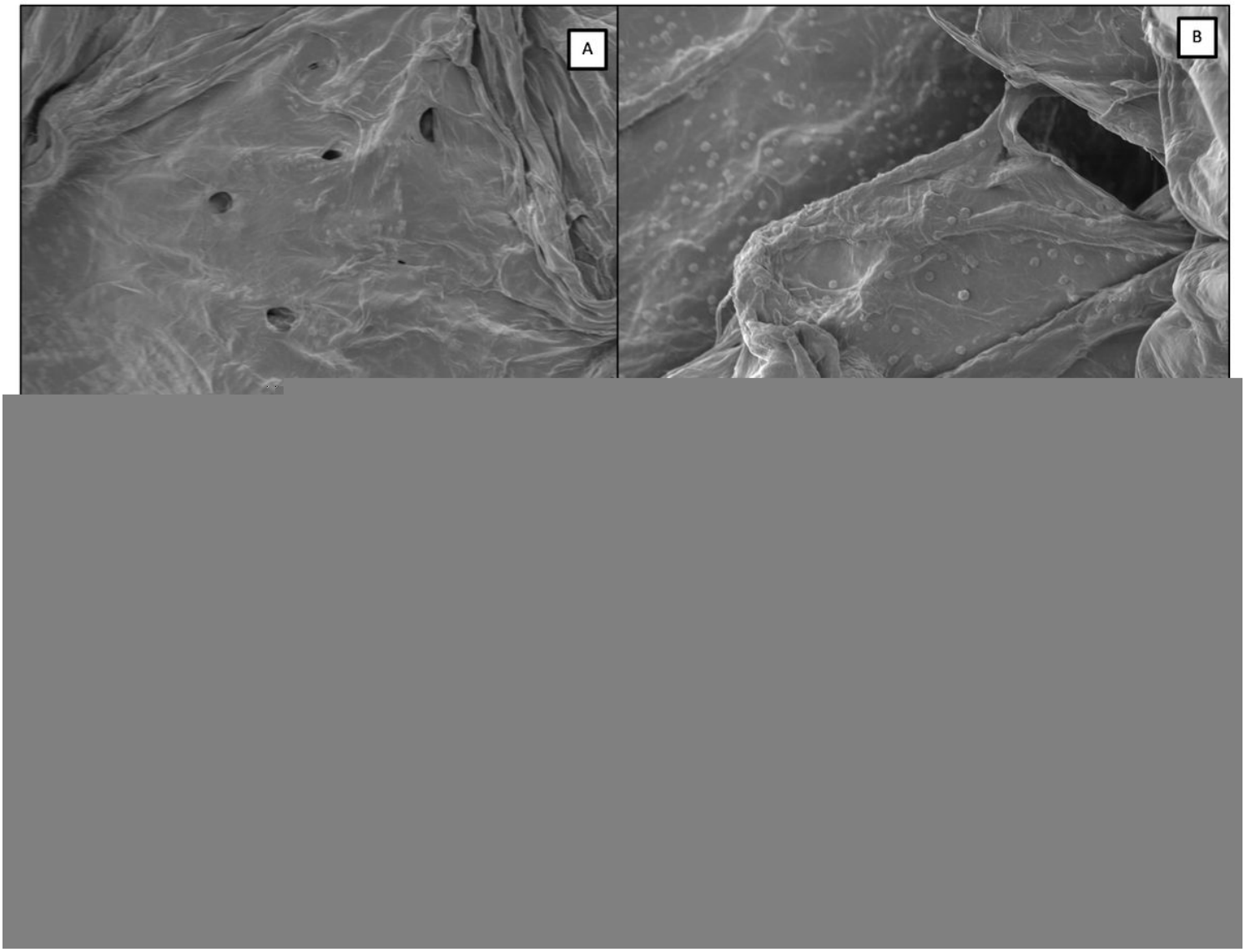

Figure 4

(A) Porous microstructure of carrageenan hydrogel. (B) 10% w/v aqueous extract of *Cissus quadrangularis* hydrogel showing evenly dispersed particles of *Cissus quadrangularis* (C) 20% w/v aqueous extract of *Cissus quadrangularis* hydrogel showing evenly dispersed dense arrangement of *Cissus quadrangularis* particles. (D) 30% w/v aqueous extract of *Cissus quadrangularis* hydrogel showing clumping of *Cissus quadrangularis* particles.

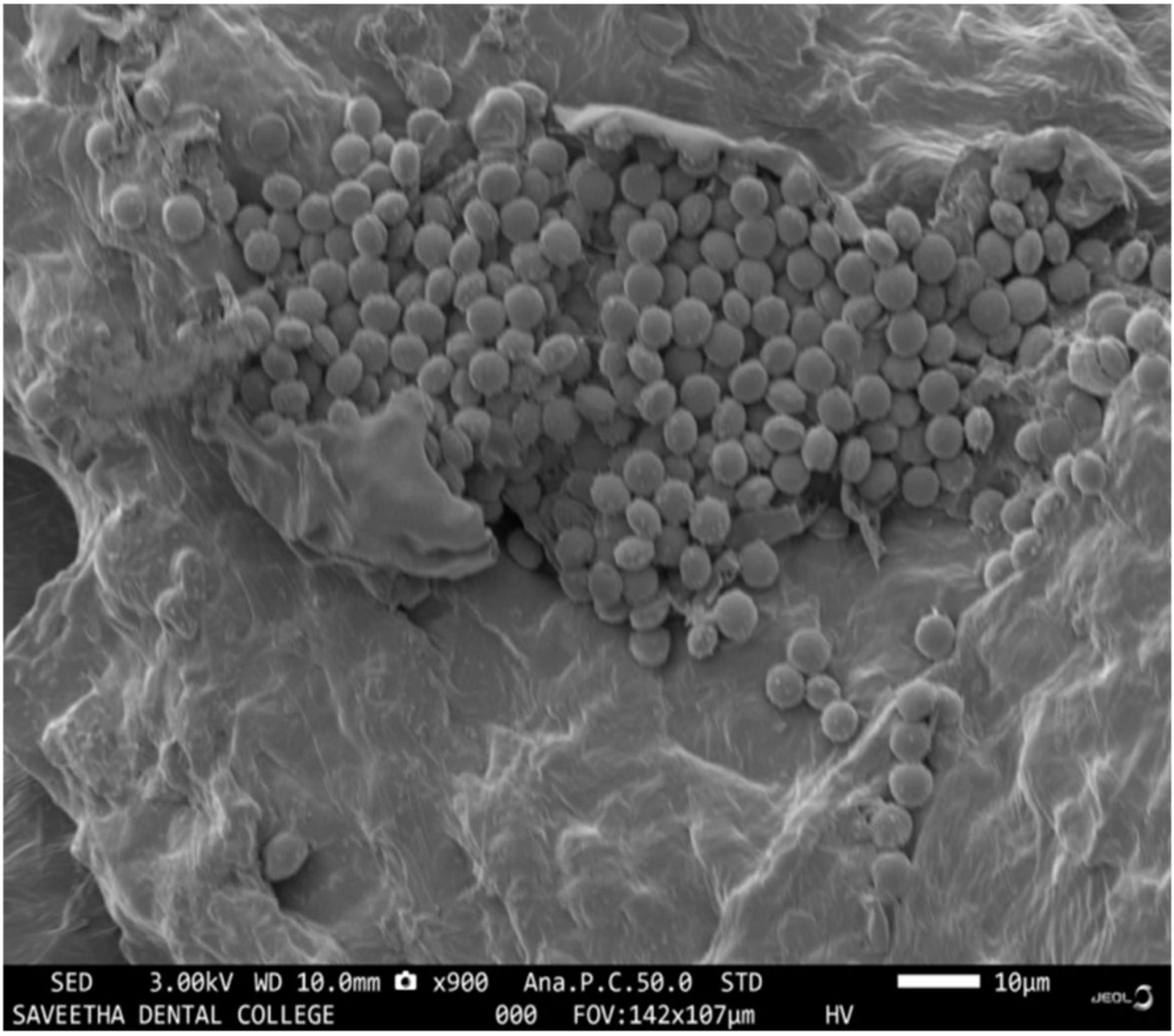

Figure 5

Higher magnification view of 30 % w/v *Cissus quadrangularis* hydrogel (Group IV) with evident clumping of *Cissus quadrangularis* particles.