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Tensile Strength, Cellular Content and Degradation properties in three generations of concentrated growth factors

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Abstract

Background Concentrated growth factors (CGF) is a biomaterial with regenerative potential, enriched with platelets, leukocytes, growth factors, and fibrin, but it degrades within 2–3 weeks. Albumin extends CGF stability, while silver nanoparticles (SNP) improve its mechanical and antibacterial properties. This in vitro and ex vivo study investigates the impact of albumin (Alb-CGF) and albumin with SNP (Alb-CGF-SNP) on CGF's mechanical properties, degradation rate, and cellular bioactivity.

Methods Blood samples were collected from 15 healthy volunteers who met specific inclusion criteria, with the sample size determined using G*Power software for power calculation. Three groups were prepared: control CGF, experimental Alb-CGF, and Alb-CGF-SNP. Membranes were produced using a Medifuge MF200 centrifuge and activated plasma albumin gel (APAG) device following standard settings. In experimental groups, the superficial 2.5 ml of plasma layer was heated at 75 °C for 10 min before combining with the buffy coat layer of CGF. Mechanical properties were tested using a texture analyzer, degradation rates were measured by weight loss percentage, and cellular bioactivity was evaluated with a Sysmex hematology analyzer. Data analysis was conducted using GraphPad Prism 8.0. Group differences were assessed via one-way ANOVA and Welch ANOVA, with Tukey's HSD test for post hoc paired group comparisons.

Results The control (CGF) showed the highest mechanical properties, with Ultimate Tensile Strength (UTS) (95.6 kPa), Modulus of Toughness (55.55 kJ/m^3), and Young's Modulus (75.73 kPa; ($P < 0.01$). No significant differences were observed in the strain at break across groups ($P > 0.90$). Alb-CGF-SNP displayed superior degradation resistance, with 45.2% weight loss at day 60 versus 84.2% in CGF ($P < 0.01$). CGF had the highest WBC and platelet levels, with amounts of 2.25 and 3.11-fold, respectively ($P < 0.01$).

Conclusion The modification of CGF with albumin and silver nanoparticles enhanced degradation resistance, although it did not reach the tensile strength and cellular content of unmodified CGF. Clinically, Alb-CGF and Alb-CGF-SNP serve as effective barrier membranes due to their prolonged stability, while CGF remains advantageous where high mechanical strength is required. Despite lower elasticity limiting suturing, their plasticity supports use as fillers or for tissue phenotype modification in regenerative applications.

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Keywords Concentrated Growth Factors (CGF), Albumin, Silver Nanoparticles (SNP), Cell Count, Tensile Strength, Biodegradation

Introduction

Autologous biomaterials remain the most utilized in regenerative dentistry due to their superior properties, including osteoinduction, osteoconduction, and osteogenesis, while minimizing the risk of infection, thus maintaining their status as the "gold standard" [1]. These materials can be derived from various sources, including hard and soft tissues (bone and connective tissue) or blood. Autologous platelet concentrates (APCs) have evolved through distinct generations. The first generation, predominantly represented by platelet-rich plasma (PRP), involved the use of anticoagulants (e.g., sodium citrate, EDTA) and additional components (e.g., calcium chloride, bovine thrombin) in the collection process, requiring two centrifugation steps [2]. Although PRP demonstrated utility in certain clinical settings, its outcomes were inconsistent. Other early APCs, such as fibrin glues, platelet gels and plasma rich in growth factors (PRGF) were developed [3]. PRGF, requiring both an anticoagulant and calcium chloride to activate platelets, resulted in growth factor release [4]. However, the PRGF protocol suffered from reproducibility issues, potentially leading to undesirable tissue responses [4–6].

The second generation of APCs, introduced in 2001, represented a cost-effective, user-friendly alternative with improved clinical outcomes. Leukocyte-platelet-rich fibrin (L-PRF®), the key representative of this generation, formed a superior scaffold without the need for added compounds [1, 7, 8].

Concentrated growth factors (CGF), a second-generation platelet concentrate similar to platelet rich fibrin (PRF), is produced by modifying centrifugation speeds (2400 rpm to 3000 rpm) and durations. CGF is characterized by a rigid fibrin structure rich in growth factors, accelerating cell proliferation and differentiation [9–12]. This generation of APCs, with their three-dimensional scaffolds, promotes a sustained release of cytokines and growth factors, such as transforming growth factors (TGF), platelet-derived growth factors (PDGF), vascular endothelial growth factors (VEGF), and insulin-like growth factors (IGF), which significantly enhance the early phases of tissue repair, over 10 days [2, 5, 12, 13]. In contrast, the first-generation PRP is fully dissolved within 3 days, releasing its growth factors in the initial hours [1, 14].

Many techniques have been employed to optimize the mechanical and antibacterial properties of APCs, such as SNP, which are widely used in contemporary medical applications due to their notable biocompatibility and beneficial characteristics, particularly their antimicrobial properties [15]. Research has shown that SNP are effective against a broad range of Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains, and they also exhibit antifungal and antiviral properties [16]. In this study, silver nanoparticles (SNP) were incorporated into CGF to enhance its antimicrobial properties while preserving its mechanical integrity. Inorganic antimicrobial agents have gained increasing attention in recent years due to their improved safety and long-term stability. Existing research highlights their high biocompatibility alongside favorable physicochemical properties, rendering them suitable for various biomedical applications [17]. While the antibacterial effects of SNP are well-documented [17], there remains a gap in the literature regarding their influence on mechanical properties, cellular content, and degradation characteristics. No recent studies have comprehensively examined these aspects, necessitating further investigation.

Moreover, the use of serum albumin in tissue engineering is well-documented [18]. Albumin, a highly abundant and easily isolated protein from human plasma, provides a biocompatible scaffold for cell proliferation, and biomaterials enriched with albumin have demonstrated improved dimensional stability, indicating slower degradation in vitro [18]. Additionally, research has shown that the incorporation of albumin can modulate the ultrastructure and permeability of the fibrin network, resulting in thicker fibers and a coarser nodular structure [19]. These findings suggest that integrating denatured serum albumin could enhance the CGF-based framework, creating a fully autologous, biocompatible material with potentially greater durability and prolonged therapeutic action.

CGF membrane is recognized for its enhanced mechanical properties, such as high tensile strength and favorable handling, particularly regarding suturability. However, these advantages are short-lived, typically persisting for no more than two weeks. This has led to ongoing efforts to develop CGF membranes that exhibit greater resistance to degradation over an extended period. Despite the significance of this pursuit, there remains a distinct lack of research on the mechanical and biological properties of ALB-CGF and ALB-CGF-SNP.

Therefore, this study aimed to investigate the mechanical, cellular content and degradation characteristics of ALB-CGF and ALB-CGF-SNP and compare them to CGF.

The hypothesis of the study

This study hypothesizes that the incorporation of ALB and SNP into CGF enhances its mechanical properties, cellular content, and degradation resistance, with ALB-CGF and ALB-CGF-SNP exhibiting superior tensile strength, increased cellular content, and improved degradation resistance compared to CGF alone. The null hypothesis states that there is no significant difference in tensile strength, cellular content, or degradation resistance between CGF-ALB, CGF-ALB-SNP and CGF, or that the inclusion of ALB and SNP may result in similar or inferior mechanical and biological properties compared to CGF alone.

Methodology

This in vitro study examined various ex vivo derived CGF membranes with and without albumin and SNP. A carefully controlled selection process ensured participant eligibility based on health and safety criteria. All procedures adhered to ethical guidelines and blood samples were collected systematically for further analysis.

Participants selection

The sample size was determined using G*Power software, incorporating a statistical power of 0.95, a significance level (α) of 0.05, and an effect size of 0.75, derived from preliminary experimental findings. Fifteen participants were recruited following this calculation and were required to complete a comprehensive self-reported medical history questionnaire, utilizing a standardized medical history template. Strict ethical protocols ensured voluntary participation, and eligibility was assessed according to predefined inclusion and exclusion criteria (Appendix Table 1).

Participant consent

Informed consent was obtained before blood collection, ensuring participants were fully aware of the study's purpose, significance, and potential risks. Detailed consent forms and participant information sheets provided all study specifics, including the right to withdraw at any time. Blood donation records were reviewed to adhere to safe donation limits. Confidentiality was strictly maintained, with secure data storage throughout and destruction upon study completion. Ethical approval was granted by Ulster University's Research Ethics Committee (REC/23/0072) and the College of Medicine and Dentistry.

Preparation of CGF, ALB-CGF and ALB-CGF-SNP samples

Blood samples were collected in the Blood Lab at the Center of Molecular Biosciences, within the Human Intervention Studies Unit (HISU) at Ulster University, from 15 healthy eligible volunteers, with each providing six 9 mL tubes and one 4 mL BD Vacutainer® K2 EDTA tube for blood counts. Two VACUETTE® 9 mL Serum Clot Activator plastic tubes with red caps (Greiner Bio-One, Austria) were designated for CGF preparation. Moreover, four VACUETTE® 9 mL Serum plastic tubes with white caps (Greiner Bio-One, Austria) were allocated for ALB-CGF and ALB-CGF-SNP.

All six tubes were centrifuged simultaneously in the Medifuge MF200 (Silfradent, Srl, Forli, Italy), with red-capped and white-capped plastic tubes positioned opposite each other for balance. Cycle parameters:

- Acceleration: 30 s
- 2 min at 2,700 rpm (500 g)
- 4 min at 2,400 rpm (400 g)
- 4 min at 2,700 rpm (500 g)
- 3 min at 3,000 rpm (600 g)
- Deceleration: 30 s

The total centrifugation time was 14 min. This cycle effectively separated the blood into three layers: platelet-poor plasma (PPP) at the top, concentrated growth factors (CGF) in the middle, and red blood cells (RBCs) at the bottom. The CGF gel was carefully extracted from the RBC layer using sterile forceps and scissors, ensuring proper separation. The buffy coat was included to maximize WBC and platelet yield and was then transferred to a sterile environment for further processing.

For ALB-CGF preparation from two plain white-capped plastic tubes. After centrifugation, 2.5 ml of PPP was drawn using a syringe fitted with an 18 G needle. This plasma was denatured by placing the syringe in the APAG® system (Silfradent, Italy) at 75 °C for 10 min, followed by cooling in a water bath at 2–4 °C for an additional 10 min. The buffy coat layer was combined with the denatured plasma, and fibrin polymerization was completed within 5 min, forming the Alb-CGF clot.

The recommended concentration range for silver nanoparticles in human applications typically falls between 0.001 percent and 0.1 percent weight/weight, corresponding to a concentration of 0.01 to 1 mg/ml.

Silver nanoparticles are widely incorporated into various products approved by the United States Food and Drug Administration (United States Food and Drug Administration, n.d.) [20]. For the ALB-CGF-SNP preparation, 1 ml of 0.2 mg/ml SNP, prepared from silver nitrite, sodium borohydride, and fully saturated sodium chloride, was added to each tube before centrifugation, and the tubes were inverted 8–10 times and placed on a rotator for continuous mixing of blood samples for 1 min to ensure homogenous distribution of the SNP. Following centrifugation, the 2.5 ml of PPP was denatured in the APAG® system, similar to ALB-CGF. The buffy coat fraction was collected with a syringe and mixed with the denatured PPP, completing fibrin polymerization within 5 min to form the Alb-CGF-SNP clot.

Following weight standardization to 2 g per sample using a digital scale and a sterile kit, samples were stored at −80 °C until further use. All procedures were conducted under sterile conditions and following the manufacturer's protocols to maintain sample integrity, as elaborated in Appendix Fig. 1.

Mechanical study

A total of 15 samples were prepared for each of the three experimental groups: CGF, Alb-CGF, and Alb-CGF-SNP. Mechanical testing was conducted using the TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). The system was initialized with Texture Expert software for data collection. The probe height was set to 10 mm as the inter-probe distance, and a 5 kg load cell was used for calibration. A 5 kg pre-load was applied transiently to standardize the setup, then removed before testing. No external force was exerted on the samples during tensile testing; force measurements were derived exclusively from the calibrated system. Texture analyzer settings included:

- Pre-Test Speed: 1.0 mm/s
- Test Speed: 0.5 mm/s
- Post-Test Speed: 1.0 mm/s
- Total Distance: 20.0 mm
- Force Unit: Newtons
- Trigger Force: 0.10 N (Auto-trigger for biological samples)

CAD-designed polymer molds were 3D-printed, autoclaved, and quality-checked. CGF samples stored at −80 °C were defrosted and molded for uniformity.

The samples were measured and set to standardized dimensions of width: 10 mm, length: 15 mm, and 1 mm depth for the whole samples. The samples were securely mounted in the TA-XT2 Texture Analyzer, and tensile tests were performed under controlled ambient conditions of 25 ± 3 °C and 50 ± 25% relative humidity. The force and displacement data were recorded automatically by Texture Expert software and subsequently exported for detailed analysis, as elaborated in Appendix Fig. 2.

Stress–strain data were analyzed to generate stress–strain graphs using Excel. Stress values were determined by dividing the force by the initial cross-sectional area of the tissue sample, assuming a rectangular geometry. As outlined in the Handbook of Polymer Testing [21], Young's modulus is defined as the modulus of elasticity under tension, calculated as the ratio of the change in stress to the change in strain (stress/strain). For this study, the initial slope of the stress–strain curve was used to estimate Young's modulus. Tensile strain at break refers to the strain corresponding to the maximum tensile stress before sample failure, assuming no yielding occurs. Ultimate tensile stress represents the maximum stress sustained by the specimen in the tensile test, indicating its tensile strength. Toughness, or the material's capacity to absorb energy and undergo deformation before breaking, was calculated as the area under the stress–strain curve to evaluate the mechanical characteristics of the CGF samples.

Cell count and increasing factor

Blood samples from 15 participants were collected in 4 mL EDTA tubes (pink cap) as baseline controls. Using fluorescence flow cytometry, the Sysmex XN-550 Hematology Analyzer (Sysmex Corporation, Kobe, Japan) was used to quantify cell counts and assess the cell increase factor in CGF samples. Sixty samples were processed for complete blood count (CBC) analysis. Initial measurements included counts of total leukocytes, red blood cells, platelets, monocytes, lymphocytes, neutrophils, eosinophils, and basophils. After three CGF generations were prepared, samples were carefully removed from the collection tubes, and normal saline was added to each tube to reconstitute the original blood volume, with gentle mixing to achieve homogeneity. Final cell counts were then recorded using the Sysmex analyzer, elaborated in Appendix Fig. 3.

The cell count in the CGF samples was determined by subtracting the number of cells in the diluted remaining blood from the total cell count of the whole blood sample. To ensure consistency, the sample volumes were standardized to match the original blood volume before analysis in each single tube. The increasing factor, reflecting the increase in cellular concentration after centrifugation, was calculated by dividing the CGF sample cell count by the initial whole blood cell count and then multiplied by the volume ratio between the sample size and

whole size. This ratio quantitatively measured the cellular content achieved by preparing CGF samples.

Degradation assessment

In this study, 36 samples were analyzed, including 12 samples from each group of CGF generations. While the rest of the samples were used for training and calibration purposes.

Upon retrieval from the -80 °C freezer, the samples were thawed at room temperature. After defrosting, each sample was weighed using a digital balance to ensure a consistent mass of 2 g. Each sample was subsequently immersed in 8 mL of non-glucose Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Thermo Fisher Scientific, USA) to create a standardized medium for degradation assessment.

The samples were then incubated at 37 °C in a humidified atmosphere containing 5% CO_2, simulating physiological conditions. The incubation was performed using the Galaxy S CO_2 incubator (RS Biotech, Irvine, United Kingdom), which provides precise environmental control for optimal sample incubation conditions. Degradation measurements were taken at five specific time points: 1 day, 7 days, 14 days, 30 days, and 60 days, elucidated in Appendix Fig. 4.

The degradation percentage for each sample was calculated using the following equation:

$$\text{Degradation Percentage} = [(\text{Initial Sample Mass} - \text{Mass at Time Interval})/\text{Initial Sample Mass}] \times 100$$

The initial sample mass was standardized at 2 g, and the mass at each time point was recorded using a digital balance. This calculation allowed for determining the degradation percentage at each time point, facilitating the comparison of degradation rates across the CGF, Alb-CGF, and Alb-CGF-SNP groups.

Statistical analysis

Data analysis was performed with GraphPad Prism 8.0 (Inc., La Jolla, CA, USA), using one-way ANOVA and Welch ANOVA to assess group differences (mean ± SD, $P < 0.050$). For large samples (> 30), parametric tests are suitable even with normality assumption violations [22]. Kolmogorov–Smirnov confirmed non-normality in most data, while Levene's test showed mixed homogeneity results. Both Welch and one-way ANOVA were conducted, yielding consistent outcomes and affirming ANOVA's convenience for large groups. Following the one-way ANOVA, Tukey's Honestly Significant Difference (HSD) test was employed to conduct post hoc comparisons between paired groups.

Results

Mechanical study

One-way ANOVA (analysis of variance) was used to compare the mechanical properties between the groups. The study provided F-statistic, *p*-values and 95% confidence intervals (CI) were calculated for each group.

The CGF membrane exhibited the highest mechanical properties across Ultimate Tensile Strength (UTS), Modulus of Toughness, and Young's Modulus, showing significant statistical differences when compared to the other groups ($P < 0.010$ for each). In contrast, the lowest values for these three mechanical *p*roperties were observed in the Alb-CGF membrane.

No significant difference was found among the three CGF-based membranes for strain at break, with $P > 0.900$. Additionally, there was no statistically significant difference between the two experimental groups, Alb-CGF and Alb-CGF-SNP, across all mechanical properties, with $P = 0.370$, 0.550, 0.970, and 0.600 for Young's Modulus, UTS, Strain at Break, and Modulus of Toughness, respectively elaborated in Fig. 1 and Table 1.

Cell count and increasing factor

This experiment compares total WBCs, including monocytes, lymphocytes, neutrophils, eosinophils, and basophils, along with red blood cells (RBCs), platelet counts, and their respective increasing factors (IF) across four groups: CGF, Alb-CGF, Alb-CGF-SNP, and whole blood.

The following values represented cell count and increasing factor: In the CGF group, the mean WBC count*10^9/L was 11.05 and 2.25, respectively, while platelet count*10^9/L reached 776.4 and 3.11. Monocyte levels averaged 1.44 and 3.06, lymphocytes 5.23 and 2.82, neutrophils 4.45 and 1.70, eosinophils 0.14 and 1.24, and basophils 0.06 and 2.40. These findings demonstrated statistically significant differences in WBC, platelet, and specific leukocyte counts between the CGF group and other groups ($P < 0.010$), as elucidated in Table 2 and Fig. 2.

In contrast, the RBCs content demonstrated no statistically significant differences across the study groups. The mean RBC count*10^12/L was 2.09 for the CGF group, 1.41 for the Alb-CGF group, and 1.75 for the Alb-CGF-SNP group, with corresponding final increasing factors of 0.41, 0.31, and 0.38. Although slight numerical variations were observed, statistical analysis indicated no significant difference between the groups ($P > 0.050$), suggesting comparable RBCs retention among the CGF, Alb-CGF, and Alb-CGF-SNP membranes. However, a statistically

Table 1 Tensile strength comparison between CGF, Alb-CGF, and Alb-CGF-SNP membranes

Group	Group I (CGF) *N* = 15		Group II (Alb-CGF) *N* = 15		Group III (Alb-CGF-SNP) *N* = 15		F-test	*P* (ANOVA)	*P* (I- II)	*P* (I- III)	*P* (II- III)
Parameter	Mean ± SD	95%CI	Mean ± SD	95%CI	Mean ± SD	95%CI					
Young Modulus (kPa)	75.73 ± 44.43	[51.13–100.3]	30.50 ± 15.62	[21.85–39.15]	45.17 ± 19.94	[34.13–56.12]	9.16	0.000	0.000	0.010	0.370
UTS (kPa)	95.6 ± 55.82	[64.68–126.5]	24.52 ± 12.48	[17.61–31.43]	37.63 ± 18.22	[27.54–47.72]	17.86	0.000	0.000	0.000	0.550
Strain at Break %	1.01 ± 0.28	[0.85–1.17]	0.98 ± 0.34	[0.79–1.18]	0.96 ± 0.34	[0.77–1.15]	0.08	0.910	0.980	0.910	0.970
Modulus of Toughness kJ/m^3	55.55 ± 26.51	[40.87–70.24]	12.83 ± 8.15	[8.31–17.35]	18.91 ± 11.94	[12.30–25.53]	26.35	0.000	0.000	0.000	0.600

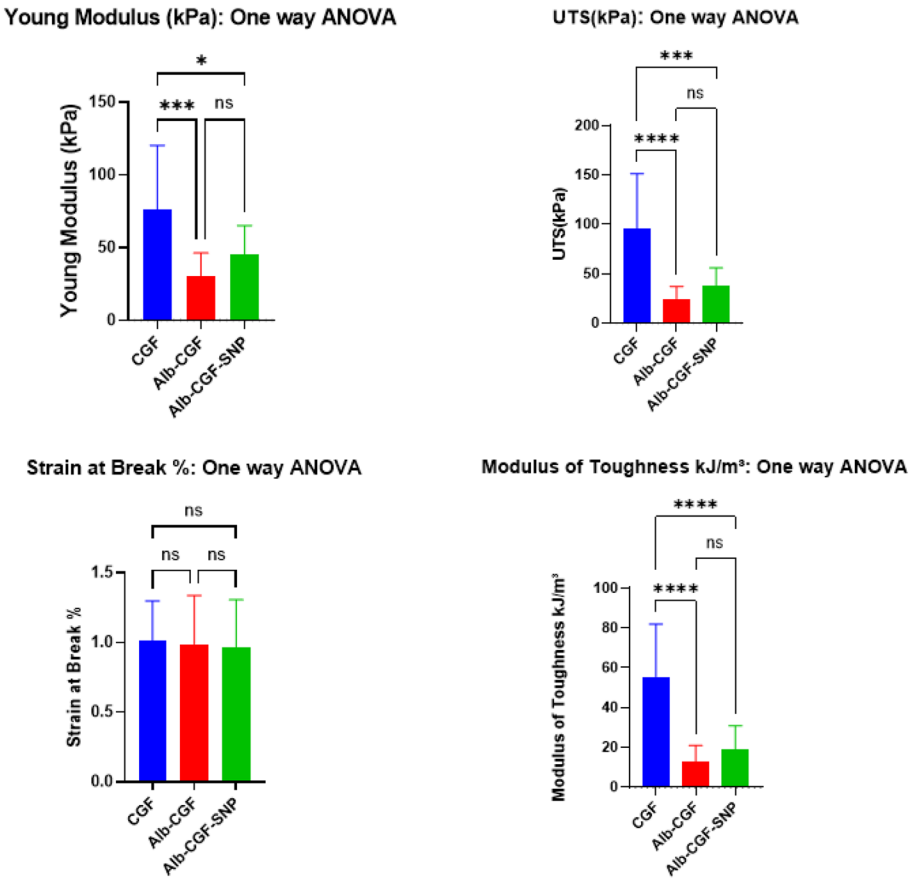

Fig. 1 Tensile strength outcomes of CGF, Alb-CGF, and Alb-CGF-SNP membranes, including Young's Modulus, UTS, Toughness, and Strain at Break. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

significant difference was observed compared to the whole blood count across all groups.

Degradation analysis

Twelve samples were prepared for the three experimental groups: CGF, Alb-CGF, and Alb-CGF-SNP. The samples were incubated in DMEM at 37 °C with 5% CO_2 at varied intervals.

The results show that the CGF group demonstrated the highest degradation rate, with a statistically significant difference compared to the other groups. The mean degradation percentages for CGF from day 1 to day 60 were as follows, respectively: 13.34%, 32.44%, 46.55%, 66.71%, and 84.20%, with a $P < 0.010$ for both comparisons with Alb-CGF and Alb-CGF-SNP.

In contrast, there was no significant statistical difference between the Alb-CGF and Alb-CGF-SNP groups, which exhibited lower degradation rates. The Alb-CGF-SNP group showed the lowest degradation after two months, with a mean value of 45.21%, as elaborated in Appendix Fig. 5 and Table 3.

Discussion

This study aimed to investigate CGF modifications by incorporating albumin (Alb-CGF) and silver nanoparticles (Alb-CGF-SNP), focusing on their mechanical properties, cellular content, and degradation characteristics. The findings provide insights into the potential therapeutic advantages and limitations of Alb-CGF and Alb-CGF-SNP, particularly in extending membrane durability for clinical use.

CGF membranes displayed the highest tensile UTS, Toughness, and Young's Modulus in mechanical property assessments, suggesting that the unmodified CGF possesses inherently superior tensile mechanical properties. The addition of albumin to CGF did not enhance these properties; instead, it resulted in a significant decline, weakening the tensile strength properties. While slightly improving the mechanical properties over Alb-CGF, SNP did not yield statistically significant enhancements. The addition of SNP offers a slight improvement over albumin alone, reinforcing the CGF

Table 2 Cell counts and increasing factors for total and subtypes of WBCs, RBCs and PLT across CGF, Alb-CGF, Alb-CGF-SNP, and WB samples

Group	Group I CGF *N*=15				Group II Alb-CGF *N*=15				Group III Alb-CGF-SNP *N*=15		Group IV (WB) *N*=15	
Parameter	Mean ±SD		95%CI		Mean ±SD		95%CI		Mean ±SD	95%CI	Mean±SD	95%CI
WBC Count *10^9/L	11.05±3.22		[9.27-12.84]		3.54±2.06		[2.39-4.68]		3.75±1.41	[2.96-4.53]	4.94±1.37	[4.18-5.70]
RBC Count *10^12/L	2.09±0.91		[1.58-2.60]		1 1.41±0.28		[1.25-1.57]		1.75±0.78	[1.32-2.19]	4.50±0.40	[4.28-4.73]
PLT Count *10^9/L	776.4±158.9		[688.4-864.4]		302.5±111.3		[240.9-364.2]		334.1±111.5	[272.3-395.8]	256.6±74.7	[272.3-395.8]
NEUT Count *10^9/L	4.45±2.30		[3.18-5.73]		1.39±1.37		[0.63-2.15]		1.38±0.69	[1.00-1.77]	2.60±1.15	[1.96-3.24]
LYMPH Count *10^9/L	5.23±1.87		[4.19-6.27]		1.69±0.94		[1.17-2.22]		1.90±0.87	[1.42-2.38]	1.82±0.49	[1.55-2.10]
MONO Count *10^9/L	1.44±1.09		[0.83-2.04]		0.55±0.75		[0.13-0.97]		0.56±0.73	[0.15-0.97]	0.50±0.51	[0.22-0.79]
EO Count *10^9/L	0.14±0.13		[0.07-0.22]		0.05±0.04		[0.03-0.08]		0.06±0.04	[0.03-0.08]	0.10±0.06	[0.07-0.14]
BASO Count *10^9/L	0.06±0.04		[0.04-0.09]		0.02±0.02		[0.00-0.04]		0.02±0.02	[0.01-0.04]	0.02±0.01	[0.01-0.03]
Group	F-test		*P* (ANOVA)		*P* (I-II)		*P* (I- III)		*P* (I-IV)	*P* (II-III)	*P* (II-IV)	*P* (III-IV)
WBC	40.58		0.000		0.000		0.000		0.000	0.970	0.290	0.430
RBC	69.26		0.000		0.030		0.490		0.000	0.470	0.000	0.000
PLT	62.85		0.000		0.000		0.000		0.000	0.880	0.710	0.280
NEUT	13.94		0.000		0.000		0.000		0.000	0.990	0.130	0.130
LYMPH	32.48		0.000		0.000		0.000		0.000	0.960	0.990	0.990
MONO	4.75		0.000		0.010		0.020		0.010	0.990	0.990	0.990
EO	3.95		0.010		0.020		0.030		0.590	0.990	0.330	0.390
BASO	40.58		0.000		0.000		0.000		0.000	0.990	0.290	0.430
Group	Group I Final I.F (CGF) *N*=15		Group II Final I.F (Alb-CGF) *N*=15		Group III Final I.F (Alb-CGF-SNP) *N*=15		F-test		*P* (ANOVA)	*P* (I-II)	*P* (I- III)	*P* (II-III)
Parameter	Mean ±SD 0(-fold over control)	95%CI	Mean ±SD (-fold over control)	95%CI	Mean ±SD (-fold over control)	95%CI						
WBC	2.25±0.42	[2.01-2.48]	0.69±0.28	[0.53-0.84]	0.76±0.25	[0.62-0.90]	107.68		0.000	0.000	0.000	0.790
RBC	0.41±0.16	[0.32-0.50]	0.31±0.06	[0.27-0.35]	0.38±0.14	[0.30-0.46]	2.14		0.130	0.120	0.840	0.320
PLT	3.11±0.56	[2.79-3.42]	1.16±0.18	[1.06-1.26]	1.30±0.26	[1.15-1.44]	125.7		0.000	0.000	0.000	0.580
NEUT	1.70±0.46	[1.45-1.96]	0.49±0.25	[0.35-0.63]	0.56±0.24	[0.42-0.69]	61.75		0.000	0.000	0.000	0.850
LYMPH	2.82±0.42	[2.59-3.06]	0.90±0.36	[0.70-1.10]	1.02±0.30	[0.85-1.19]	126.7		0.000	0.000	0.000	0.640
MONO	3.06±0.48	[2.79-3.33]	0.94±0.34	[0.74-1.13]	1.01±0.35	[0.81-1.21]	135.4		0.000	0.000	0.000	0.870
EO	1.24±0.65	[0.87-1.60]	0.58±0.38	[0.37-0.80]	0.57±0.31	[0.40-0.75]	9.53		0.000	0.000	0.000	0.990
BASO	2.40±0.99	[1.85-2.95]	0.74±0.59	[0.41-1.07]	0.85±0.54	[0.55-1.15]	23.70		0.000	0.000	0.000	0.900

structure without surpassing the mechanical strength of unmodified CGF.

However, regarding strain at break, the consistency among the three groups indicates that albumin and SNP modifications do not significantly affect the material's flexibility. The stability of CGF allows it to maintain high tensile strength without losing adaptability. This balance of tensile strength, elasticity, suturability, and flexibility makes CGF a viable choice for short-term regenerative applications.

In contrast, the lower elasticity of the experimental groups may limit their use in suture-based procedures. Nevertheless, similar plasticity across all groups suggests they could be effective as fillers or for altering soft tissue phenotype. Further clinical and preclinical studies are recommended to validate these findings and explore their broader applications.

The findings offer a nuanced perspective on CGF, and its modified forms compared to previous studies.

Khorshidi et al. demonstrated that SNP-modified L-PRF membranes possessed superior tensile strength and antibacterial properties, emphasizing SNP's potential to enhance structural and functional properties in platelet-rich fibrin membranes [17]. While SNP addition in this study did not significantly improve the tensile strength of CGF because of the addition of albumin with SNP, it delayed degradation, aligning with Khorshidi's observations on SNP's stabilizing effects. This degradation delay makes Alb-CGF-SNP a promising material for extended scaffold presence applications.

Regarding degradation analysis, it was revealed that CGF membranes underwent rapid degradation, with the highest breakdown rates observed by Day 60, underscoring its limitations for long-term use. In contrast, Alb-CGF-SNP exhibited the slowest degradation rates across the study, indicating that SNP addition effectively delays membrane breakdown, a feature beneficial for applications requiring prolonged support in tissue regeneration. Isobe et al. found comparable degradation rates in CGF and A-PRF without any substantial delay, without mention of SNP addition to enhance degradation resistance [11]. Zheng et al. explored the degradation and mechanical properties of PRF gels treated with heat and found that heating significantly delayed degradation and enhanced rheologic mechanical properties without compromizing cellular viability [23].

Simões-Pedro et al. compared different PRF membranes and found that A-PRF + achieved higher tensile strength and viscoelastic properties than L-PRF, highlighting the benefits of customized PRF protocols. Current findings indicate that while CGF displays better mechanical strength, SNP additions to CGF provide moderate enhancement in degradation delay without matching the high tensile properties noted in A-PRF + [24].

Moreover, Isobe et al. investigated the mechanical and degradative characteristics of Advanced-PRF (A-PRF), CGF, and PPTF membranes, concluding that PPTF displayed reduced tensile strength and increased degradation. These findings suggest that CGF offers a more robust mechanical profile than PPTF [11].

Mourão et al. explored Alb-CGF membranes, combining growth factors with denatured albumin. These membranes were moldable, stable, and uniformly cellular, releasing growth factors (PDGF, VEGF, FGF2) over seven days. Their regenerative potential suggested applications in guided tissue regeneration, warranting further research to evaluate long-term clinical outcomes and broader surgical uses [25].

Houshmand et al. demonstrated that activated plasma albumin gel undergoes significant biodegradation over 21 days, as evidenced by SEM imaging and weight reduction analysis. The substantial loss of structural integrity suggests that the degradation rate is relatively high. As a result, the study recommends using a thicker membrane compared to the conventional thickness of the connective tissue graft to compensate for biodegradation and maintain long-term stability in soft tissue regeneration [26].

Wu et al. demonstrated that thermal manipulation enhances the mechanical strength and degradation resistance of horizontal-PRF (H-PRF) membranes, particularly at temperatures above 90 °C. However, excessive heating at 105 °C significantly reduced cell viability and compromised biological effects on osteoblast proliferation. These findings suggest that while thermal treatment can improve membrane stability, it must be carefully optimized to preserve biological functionality [27]. Comparing these results with the present study highlights the importance of balancing mechanical reinforcement and bioactivity in regenerative applications. However, there is no mention of albumin incorporation and denaturation.

Cellular content analysis indicated that CGF membranes exhibited the highest concentrations of white blood cells (WBCs) and platelets compared to Alb-CGF and Alb-CGF-SNP; however, these values were lower than those reported in similar studies. For instance, Masuki et al. observed substantially more significant increases in WBC and platelet counts in CGF, potentially due to differences in analytical methodologies [28]. Although these CGF-based membranes showed elevated cellular concentrations relative to Alb-CGF and Alb-CGF-SNP, they did not reach the levels reported in previous research, suggesting possible variations in cellular quantification and preparation methods. These findings underscore the necessity of establishing a standardized

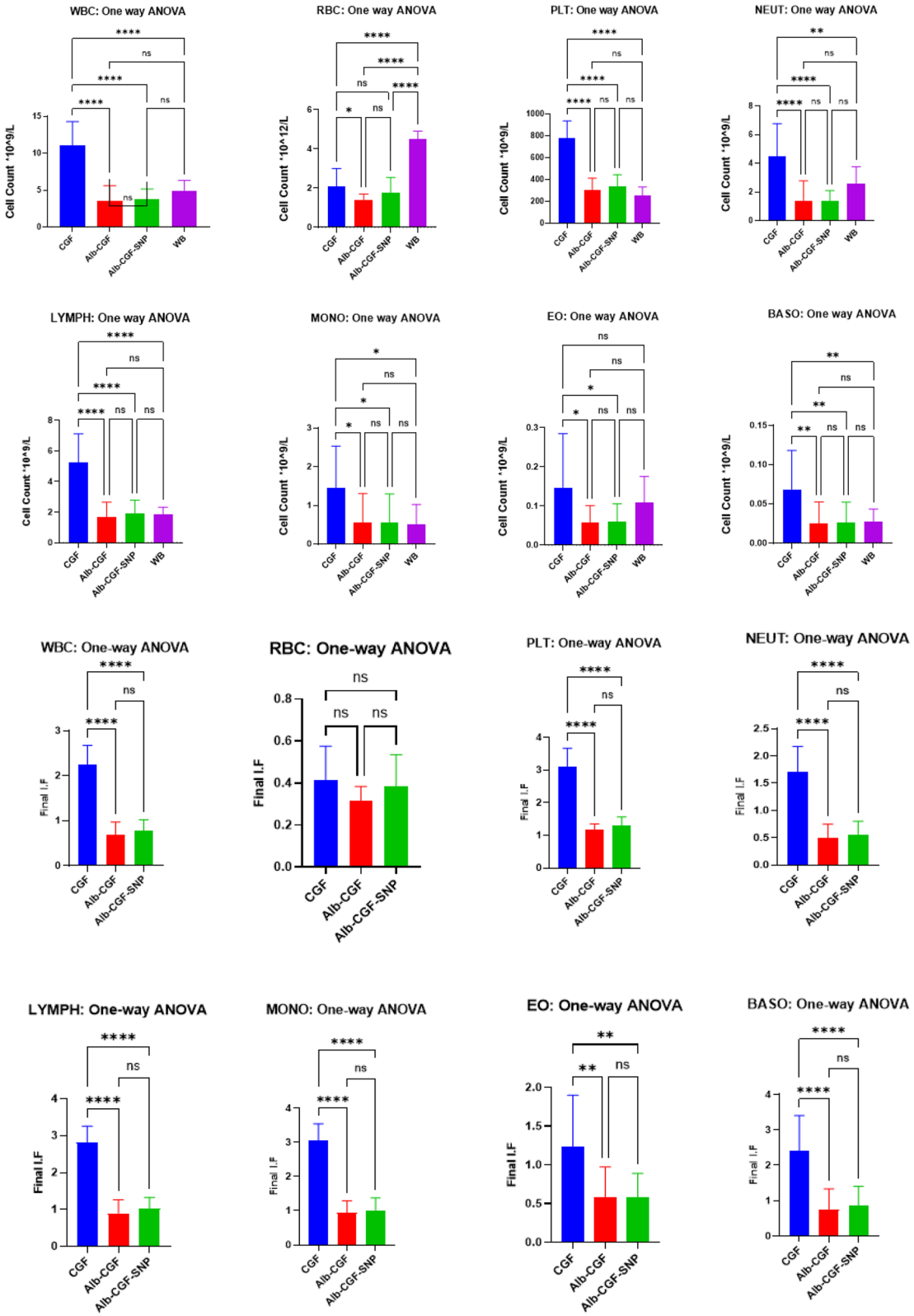

Fig. 2 Cell count and the increasing number over the control in total and subtypes of WBCs, RBCs, and PLT, in CGF, Alb-CGF, Alb-CGF-SNP, and WB samples analyzed by flow cytometry. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

Table 3 Mean degradation percentages for CGF, Alb-CGF, and Alb-CGF-SNP membranes measured at intervals (Day 1, 7, 14, 30, 60)

Group	Group I (CGF) *N* = 15		Group II (Alb-CGF) *N* = 15		Group III (Alb-CGF-SNP) N = 15		F-test	P (ANOVA)	*P* (I-II)	*P* (I- III)	*P* (II-III)
Parameter	Mean ± SD (%)	95%CI	Mean ± SD (%)	95%CI	Mean ± SD (%)	95%CI					
Day 1	13.34 ± 10.66	[6.57–20.11]	2.0 ± 4.01	[−0.55 – 4.55]	0.5 ± 3.37	[−1.64 – 2.64]	12.58	0.000	0.000	0.000	0.850
Day 7	32.44 ± 14.18	[23.43-41.45]	7.0 ± 3.62	[4.69–9.30]	11.88 ± 6.69	[7.61–16.13]	25.35	0.000	0.000	0.000	0.410
Day 14	46.55 ± 13.31	[38.09–55.0]	*9.75 ± 5.01*	[6.56–12.94]	14.58 ± 8.58	[9.13–20.04]	52.18	0.000	0.000	0.000	0.440
Day 30	66.71 ± 17.63	[55.51–77.91]	25.54 ± 15.17	[15.90–35.18]	24.17 ± 24.09	[8.85–39.48]	18.76	0.000	0.000	0.000	0.980
Day 60	84.20 ± 15.82	[74.15–94.25]	68.63 ± 31.24	[48.78–88.47]	45.21 ± 29.15	[26.68–63.73]	6.68	0.000	0.320	0.000	0.080

protocol for CGF and its derivatives to ensure reproducibility and consistent cellular profiles crucial for regenerative efficacy.

Additionally, adding a 0.3–0.5 mL of buffy coat layer in CGF preparations is expected to significantly augment cell yields, particularly in WBCs and platelets, thereby enhancing the cellular characteristics of CGF membranes. Miron et al. demonstrated that isolating this layer immediately above the red blood cell component can lead to a tenfold increase in platelet and leukocyte concentrations, providing elevated growth factor levels crucial for applications requiring intensive tissue regeneration [29].

Moreover, Miron et al. explored horizontal centrifugation as an alternative approach, achieving a 3.5-fold increase in cell concentration, nearly matching the findings of this study. This technique distributes cells more uniformly within the PRF matrix, which, while yielding slightly lower cell concentration than buffy coat isolation, may be advantageous in applications where homogeneous cellular distribution is preferred [30].

Furthermore, Miron et al. emphasized the importance of plain, additive-free glass tubes in PRF preparation, as tubes with silica or silicone coatings can negatively impact clot formation, reducing the PRF clot size and introducing contaminants that compromise cell viability and increase inflammatory response. Specifically, silica contamination has been shown to hinder cell functionality and induce apoptosis, thus diminishing the therapeutic quality of PRF preparations [31]. This study is limited by using silica-coated evacuated plastic tubes for CGF preparations, which may influence the results.

Accordingly, given the consistent g-force settings of the Medifuge MF200, it is essential to consider the effects of these variables, such as buffy coat inclusion and recommended tube material, to enhance the reliability and reproducibility of results. Such standardization facilitates meaningful comparisons with previous studies, enabling reliable cellular concentrations and optimizing the regenerative properties of CGF and related platelet-rich formulations.

A clinical study by Ntontoulos and Dabarakis demonstrated that the Albumin-Concentrated Growth Factor (Alb-CGF) protocol in minimally invasive sinus piezosurgery effectively promotes new bone formation and enhances implant stability, even in cases with residual bone heights below 6 mm. Their study reported full osseointegration within 4 to 6 months, reduced postoperative morbidity, and accelerated healing. The positive correlation between implant stability values further supports the regenerative potential of Alb-CGF, highlighting its efficacy as a viable alternative to conventional sinus augmentation [32].

This study demonstrates that while unmodified CGF provides the best mechanical support among the membranes tested, SNP-modified CGF (Alb-CGF-SNP) exhibits significant degradation resistance, supporting its application in scenarios requiring prolonged material stability. However, limitations include the absence of in vivo assessments, growth factor release analysis, and antibacterial studies. Further investigations, particularly clinical and preclinical trials, are crucial for validating Alb-CGF-SNP's full potential in regenerative applications.

Conclusion

The modification of CGF with albumin and silver nanoparticles enhanced its degradation resistance, surpassing that of albumin alone, while not achieving the tensile strength and cellular content of unmodified CGF. The extended stability of Alb-CGF and Alb-CGF-SNP suggests their suitability as barrier membranes. In contrast, CGF remains preferable for applications demanding greater tensile strength and cellular content. Although the reduced elasticity of Alb-CGF and Alb-CGF-SNP limits their suturability, their comparable plasticity supports their potential use as fillers or for tissue phenotype modification in regenerative approaches. Further in vitro studies are necessary to assess growth factor release, antimicrobial properties, and the impact of different silver nanoparticle concentrations. Additionally, preclinical and clinical in vivo investigations are required to refine their role in regenerative applications and determine their long-term efficacy.

Supplementary Information

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Supplementary Material 1.

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Informed consent

Informed consent was obtained after participants reviewed the eligibility criteria and participant information sheet. Each participant was thoroughly briefed on the study's purpose, significance, and potential risks and then signed the consent form, acknowledging their understanding and voluntary participation, including the option to withdraw at any time.

Authors' contributions

A.A. contributed to the conception and design of the study, performed the analysis, drafted the manuscript, and critically revised it. All authors contributed to the study's conception and design, critically reviewed the manuscript,

provided final approval, and agreed to be accountable for all aspects of the work, ensuring its integrity and accuracy.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval for this study was granted by the Research Ethics Committee at Ulster University (REC/23/0072) and the College of Medicine and Dentistry, ensuring compliance with ethical standards for research involving human participants. This study was conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki (1964), as revised in 2013.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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