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Research Article

Gelatin/PLGA Microspheres as a 3D Scaffold for Chondrocytes

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Abstract

Background*:** Osteoarthritis (OA) degrades cartilage and bone. Osteochondral autograft, allograft, and total replacement knee surgery have limitations, such as prompt immune responses, lack of cartilage tissue obtainability, invasiveness, and a loosening implant that may require further correction. Tissue engineering, which involves injecting chondrocytes into 3D porous scaffold carriers in the joint, seems promising for tissue repair and growth. ***Objective: To develop gelatin/poly DL-lactide-co-glycolide (PLGA) microspheres as a porous scaffold for chondrocyte carriers. ***Methods***: The double emulsion method is one of the most popular and best methods for forming microspheres. In summary, in the PLGA oil phase, we emulsified a gelatin solution representing the inner aqueous phase. Next, in an external aqueous phase of polyvinyl alcohol (PVA), we emulsified the resultant first emulsion. The double emulsion was stirred to evaporate organic solvent and centrifuged to collect gelatin and PLGA microspheres. ***Results***: The Mastersizer result showed polydispersed particles with 23.53% of the desirable cell injection size range between 1-300 µm. Scanning electronic microscope (SEM) images revealed spherical and porous microspheres with smooth surfaces. The average absolute zeta potential value was -30.7±4.895, indicating stable preparation. ***Conclusions***: Gelatin and PLAGA polymers worked together to make 3D scaffold microspheres that were the right size, had the right number of holes, and were strong.

Keywords: Chondrocytes, Gelatine, Microspheres, PLGA.

الجيلاتين/PLGA المجهرية كسقالة D3 للخلايا الغضروفية

الخلاصة

الخلفية: التهاب العظام يحطم من الغضاريف والعظام. ان العلاج باستخدام الطعم الذاتي العظمي الغضروفي، والطعم الخيفي، وجراحة الركبة البديلة بالكامل لها قيود مثل الاستجابات المناعية الفورية، ونقص إمكانية الحصول على أنسجة الغضاريف، والارتخاء الذي قد يتطلب مزيدا من التصحيح. هندسة الأنسجة، والتي تنطوي على حقن الخلايا الغضروفية في ناقلات سقالة مسامية D3 في المفصل تبدو واعدة لإصلاح الأنسجة ونموها. **الهدف**: تطوير كريات مجهرية للجيلاتين/بولي DL-lactide-co-glycolide (PLGA) كسقالة مسامية لحاملات الخلايا الغضروفية. **الطرق**: طريقة المستحلب المزدوج هي واحدة من أكثر الطرق شيوعا وأفضلها لتشكيل الكريات المجهرية. باختصار، في مرحلة زيت PLGA، قمنا باستحلاب محلول جيلاتيني يمثل المرحلة المائية الداخلية. بعد ذلك، في مرحلة مائية خارجية من كحول البولي فينيل (PVA)، قمنا باستحلاب المستحلب الأول الناتج. تم تقليب المستحلب المزدوج لتبخير المذيبات العضوية والطرد المركزي لجمع الجيلاتين والكريات المجهرية PLGA. **النتائج**: أظهرت نتيجة Mastersizer جزيئات متعددة التشتت مع 23.53٪ من حجم حقن الخلايا المرغوب فيه يتراوح بين 1-300 ميكرومتر. كشفت صور المجهر الإلكتروني الماسح عن كريات مجهرية كروية ومسامية ذات أسطح ملساء. كان متوسط القيمة المطلقة لزيتا المحتملة -30.7±4.895 ، مما يشير إلى استعداد مستقر. **الاستنتاجات**: عملت بوليمرات الجيلاتين و PLAGA معا لصنع كريات مجهرية سقالة D3 بالحجم المناسب، وكانت قوية و لديها العدد الصحيح من الثقوب.

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INTRODUCTION

Osteoarthritis (OA) is a joint disease that involves the degradation of cartilage tissues and the underlying bone structure. The incidence of OA is massively rising due to the escalation in individuals' weight and age. Several factors restrict cartilage repair mechanisms, including non-dividing chondrocytes, no rapid matrix revenue, little progenitor cell supply, the absence of vascular networks, and high intensities of protease inhibitors, all of which are responsible for inhibiting efficient tissue restoration [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) and intraarticular injections of corticosteroid, hyaluronic acid, and platelet-rich plasma were used to treat knee pain in mild to moderate OA, where cartilage starts to break down and joint space gets smaller [2,3]. However, in cases of severe OA, where the articular cartilage has completely deteriorated, the joint space has vanished, and the bones of the joint are rubbing against each other, there are only three conventional methods for repairing cartilage lesions. The first

method is osteochondral autograft, which involves transplanting a slightly smaller proportion of low-weight-bearing cartilage from the patient. However, this method may lead to complications such as donor location morbidity and a lack of cartilage tissue accessibility. The second approach is osteochondral allograft, which represents the introduction of cartilage pieces from tissue banks. Similarly, this method will be limited to prompt immune responses [4]. The third approach to cartilage lesion healing involves total joint replacement surgery. Despite its high applicability, total joint replacement surgery is not the preferred approach due to its invasiveness and risk of implant loosening, which could necessitate additional correction surgery and potentially lead to inflammation and infections [5]. Researchers have developed cartilage tissue engineering as a biomaterial scaffold for chondrocyte regeneration to overcome these limitations. The things that make scaffolds good for tissue proliferation are their three-dimensional (3D) structure with the right volume, shape, and mechanical strength; the fact that they are very porous to allow living cells to attach and express themselves; and the fact that when they break down, they don't cause many immune or inflammatory responses [6]. Additionally, studies have reported several advantages of using injectable scaffold preparation, such as allowing minimal incision during transplantation, enhancing the safety of potent drugs, reducing steady-state fluctuation levels, reducing health care costs through upgraded therapy, shortening treatment periods, and reducing dosing frequency [7]. Hydrogels and microcarriers are two examples of injectable scaffolds intended for tissue regeneration. Although both provide biomolecule diffusion and cell viability, microcarriers outperform hydrogels in terms of diffusion rate, cell mobility, cell-cell interaction, and fabrication techniques. Moreover, microcarriers provide more efficient diffusion of large biomolecules compared to hydrogel networks, and their wide range of fabricated polymers facilitates easy fabrication into various forms [8]. The microsphere is an excellent injectable microcarrier. The good things about using microspheres to encapsulate chondrocytes are their shape, which lets you control the rate of drug release; the use of biodegradable polymers that don't need to be taken out surgically; and the fact that the cells made inside microspheres are strong and can handle methods of preservation like freezing and lyophilization [9]. In addition, microspheres allow the exchange of nutrients and metabolic wastes due to their large surface area-to-volume ratio, which enables fast cell expansion [10]. People prefer the microsphere scaffold over other porous scaffolds due to its ability to generate a higher cell quantity in a short period of time [11]. Furthermore, microspheres are able to produce a desirable cell injection size range [12]. Numerous synthetic and natural polymers are available for scaffolding nanoparticles. The pharmaceutical and medical fields extensively use gelatin, a hydrophilic natural polymer, for its low antigenicity, biocompatibility, high availability, low price, and biodegradable properties. Recently, the FDA has categorized gelatin as GRAS (Generally Regarded As Safe) [13]. On the other hand, poly (DL-lactide-co-glycolide) (PLGA) is a synthetic co-polymer of poly D-glycolide acid (PGA) and poly (l-lactic acid) (PLA). Their hydrophilic-hydrophobic profile depends on PLA: PGA ratio. PLGA's ability to form porous scaffolds, ease of microsphere fabrication, and biocompatible and biodegradable properties have led to its extensive use in musculoskeletal tissue engineering in bone, cartilage, and meniscus [14]. Numerous studies have confirmed the high affinity of positively charged gelatin toward damaged cartilage. This affinity stems from its attraction to the negatively charged cell membrane surface due to the phospholipid bi-layer structure [15], its attraction to the densely packed negatively charged aggrecan glycosaminoglycans present in the cartilage [16], and its ability to target fibronectin-bearing surfaces [17], a substance highly secreted by damaged cartilage with OA [18]. On the other hand, Chun and his colleagues have proven that positively charged PLGA microspheres lead to higher cell proliferation, growth and attachment compared to negatively charged PLGA microspheres [19]. The combination effect of gelatin and PLGA would enhance PLGA hydrophobicity and poor osteoconductivity. On the other hand, it would control the chondrocyte release rate from gelatin polymers [20]. The current study developed gelatin/PLGA microspheres as a 3D scaffold for chondrocyte delivery and as an injectable platform for cartilage repair.

METHODS

Materials

Poly DL-lactide-co-glycolide (PLGA; 75:25) with molecular weight 66,000–107,000 g/mol and span 80 were obtained from Nano Life Quest Sdn. Bhd. (Sigma-Aldrich, Germany). Dichloromethane was purchased from Chemolab Supplies Sdn. Bhd. Polyvinyl alcohol (PVA) (partially hydrolyzed grade) and Tween 80 were obtained from LGC Scientific Sdn. Bhd. Gelatin was a gift from the Department of Tissue Engineering, UKM Medical Centre.

Gelatin/PLGA fabrication

Gelatin/PLGA microspheres were fabricated using a double emulsion solvent evaporation technique of water/oil/water (W/O/W), as reported elsewhere [21,22] with some modifications. The schematic representation of the method is shown in Figure 1. Initially, 20 mg/ml of gelatin were hydrated using a water bath at 40 °C. On the other hand, we prepared 50 mg/mL of PLGA and DCM using a bath sonicator. Then, 5:1 v/v of emulsifier (tween 80/span 80) was added to the PLGA solution and sonicated for 15 minutes using a bath sonicator. Afterwards, we formulated the first (W/O) emulsion by emulsifying the gelatin solution in the PLGA solution using a probe sonicator. 1% PVA was prepared using a magnetic stirrer with heat. After cooling, 3% of the emulsifier (Tween 80/Span 80 5:1 v/v) was added and

stirred. Subsequently, we added the first (W/O) emulsion dropwise to the PVA solution while stirring, resulting in a W/O/W blend. We stirred the W/O/W emulsion for 24 hours at 600 rpm at room temperature to evaporate the organic solvent, and then placed it under a vacuum desiccator for 24 hours to remove the residual DCM. Later, we centrifuged the microspheres at 5000 rpm for 10 min and washed them five times with distilled water to eliminate non-reacted PVA, emulsifiers, free gelatin, and PLGA polymers. Finally, we lyophilized the collected microspheres using a freeze dryer for two days at -110 °C.

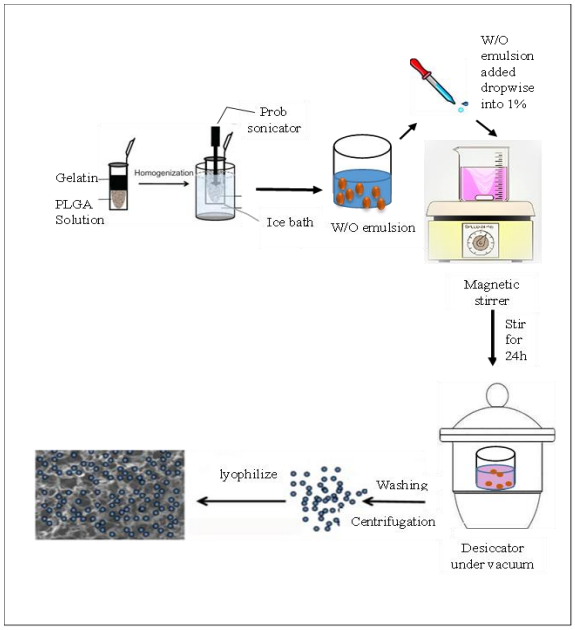

Figure 1: Schematic preparation of Gelatin/PLGA microspheres.

Mastersizer

Microspheres recovered from centrifugation were dispersed in 2 ml of distilled water and particle size was measured using a Malvern Mastersizer. Prior to analysis, the dispersed microspheres were sonicated in a bath sonicator for five minutes. The sample was introduced dropwise into the dispersion unit with a plastic pipette until the absorbance was in the range of 12–16%. The measurement was repeated three times and the particle size was reported as the average diameter.

Scanning electron microscope (SEM)

Lyophilized microspheres were sputter coated with gold and analyzed using SEM at an accelerating voltage of 10 kV. The diameter of microspheres and their porosity were viewed and recorded. The sample was also observed to inspect the uniformity of microspheres morphology and to observe any aggregates if present.

Particles charge analysis

Microspheres recovered from centrifugation were dispersed in 2 ml of distilled water and particle surface charge (as characterized by zeta potential) was measured using a photon correlation spectrometer (Malvern Zetasizer). Prior to analysis, the dispersed microspheres were sonicated in a bath sonicator for five minutes. The equipment was set at 25 °C. The samples were measured with five repeatable measurements to ensure the reproducibility of the results.

RESULTS

Table 1 displays the particle size analysis that the Mastersizer performed and replicated three times. We calculated the coefficient of variation (COV) using the following formula to ensure the validity and reliability of the results:

$$COV = (\delta/av)* 100 \ldots (1)$$

Table 1: Particle size of gelatin/PLGA microsphere

Sample	D (0.1) µm	D (0.5) µm	D (0.9) µm
Gelatin/PLGA (1)	0.118	0.739	493.452
Gelatin/PLGA (2)	0.112	0.688	517.782
Gelatin/PLGA (3)	0.113	0.725	523.011
Gelatin/PLGA average (av.)	0.114	0.718	512.443
Standard deviation (δ)	0.00324	0.0264	15.82
COV%	2.84	3.67	3.088

ISO and USP (Malvern 2007) set the acceptance range. According to ISO, the COV for D (0.5) should not exceed 3%, while the COV for D (0.1) and D (0.9) should not exceed 5%. USP acceptance criteria for COV should be below 10% for D (0.5) and below 15% for D (0.1) and D (0.9). In the current study, as the calculated COV% was within the acceptable range, the obtained results were considered valid and reliable. The Mastersizer data showed that the D (0.1), D (0.5), and D (0.9) values are important for defining the peak diameter distribution width. For example, D (0.5) is the median diameter, where 50% of the particles are below 0.718 µm, and D (0.1) is the diameter where 10% of the particles are below 0.114 µm. On the other hand, D (0.9) signifies 90% of the particles are present below 512.443 µm. Figure 2 shows the particle size distribution of gelatin/PLGA microspheres obtained from Malvern Mastersizer.

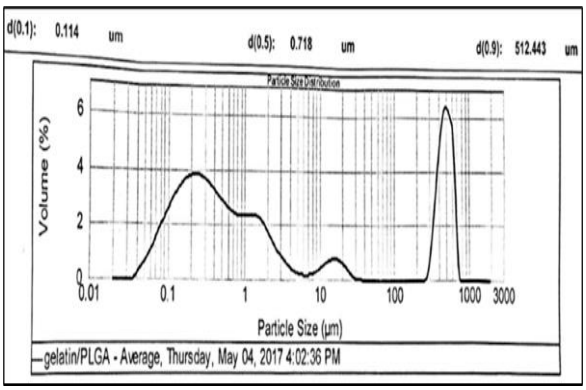

Figure 2: The size distribution of gelatin/PLGA particles.

Furthermore, Figure 3 illustrates the volume percentage of various gelatin/PLGA diameter ranges,

expressed in micrometers. It was found that the desirable size range of 1-300 µm was 23.53%.

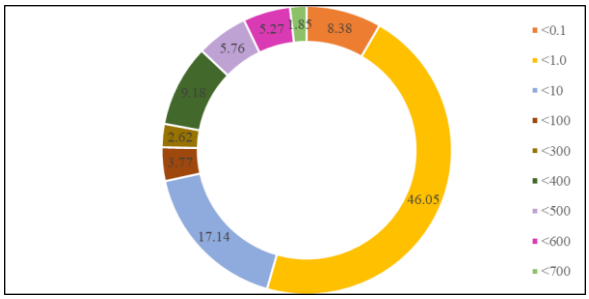

Figure 3: Represent volume % of various gelatin/PLGA diameter range in µm.

Based on observations under SEM, the gelatin/PLGA microspheres appeared to be spherical particles with smooth and porous surfaces, as shown in Figure 4.

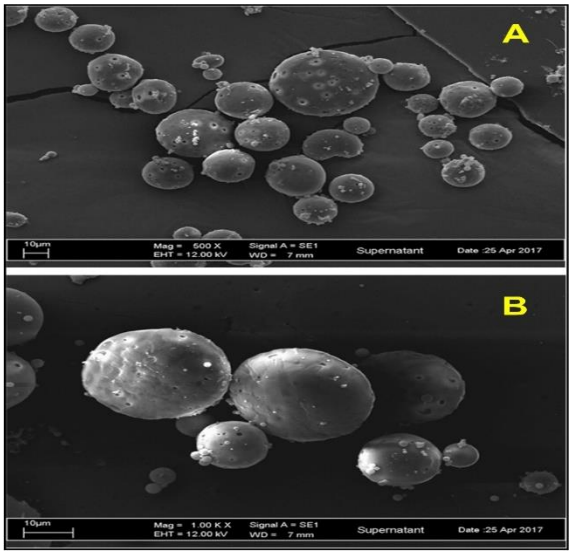

Figure 4: SEM images of gelatin/PLGA microspheres formulation at different magnifications: (A) at 5ooX; (B) at 1000X.

The porous gelatin/PLGA microspheres have diameters ranging from hundreds of nanometers up to a few micrometers, which facilitate cell growth, proliferation, and attachment, as seen in Figure 5.

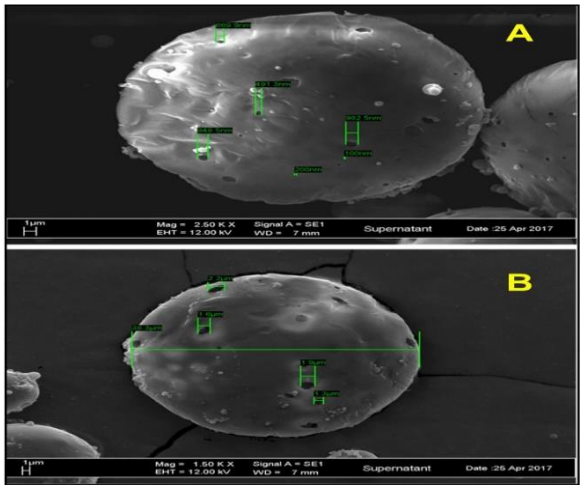

Figure 5: Porous diameter of gelatin/PLGA microspheres: (A) micron-size; and (B) nano-size pores observed on the surfaces of microparticles.

Table 2 presents the zeta potential values of two replicated samples five times.

Table 2: Zeta potential of gelatin/PLGA microparticles

Sample No.	Zeta potential (mV)	SD (mV)
Batch 1	-30.6	5.15
Batch 2	-30.8	4.64
average	-30.7	4.895

DISCUSSION

The SEM result showed a multimodal peak (multi peaks) with polydispersed particles along the x axis from 0.035 up to an 800 µm diameter range. The first peak had a broad size distribution ranging from 0.035 to 34.674 µm, while the second peak had a narrow size distribution ranging from 316.228 to 724.436 µm. We managed to obtain 23.53 percent of the prepared microspheres falling within the desired injectable size range. However, we can enhance this percentage by implementing a sieving step process following the centrifugation step, which will eliminate the unwanted large size range and produce monodisperse particles with a desirable narrow size range, as demonstrated by Tan and his colleagues [22]. The size and size distribution of particles are crucial characteristics for microsphere delivery systems, as they influence the microspheres' suitability for passing through a syringe needle and also aid in establishing a stronger correlation between the size of the microsphere and its release properties [12]. According to previous research, particles larger than 250 µm have difficulties during needle injection [23], whereas particles smaller than 20 µm increase the risk of inflammation and embolization due to the migration of small microspheres into distal organs [24], so their size range must be kept between 20 and 250 µm. the current research revealed that 23.53% of gelatin/PLGA microspheres had a diameter in the range of 1-300 µm, which is appropriate for administration with a syringe needle. Numerous factors were responsible for the smooth and porous surface of the prepared microspheres. The polymers used to fabricate microspheres have a direct impact on their morphology and size. As molecular weight increases, the resultant microspheres become larger and smoother. This is due to its high hydrophobicity, which provides a small surface area for diffusion [25]. So, the microspheres' range of sizes and smooth surface are caused by the amount and ratio of gelatin/PLGA polymers (75:25). Furthermore, the materials chosen to form gelatin/PLGA microspheres have a direct impact on their properties. DCM has gained prominence over other solvents for use as organic solvents due to its regular morphology, lower permeability, and stiffer scaffold formation [26]. In addition, evaporation of the entrapped DCM is thought to be responsible for the formation of internal cavities inside the microspheres. The technique and temperature used to control the DCM evaporation are key to determining the scaffold porosity characteristic. Previous reports indicate that high temperatures accelerate DCM evaporation, solidify microspheres quickly, and ultimately lead to less cavity formation

within microspheres [27]. In this study, however, gelatin/PLGA microspheres looked better for surfaces with a lot of pores when DCM evaporated at room temperature while being stirred continuously for 24 hours. Lastly, the amount of polymer has an effect on the porosity of microspheres. This is because high polymer concentrations make solutions that are very thick, which makes microspheres with low porosity [28]. This supported the current study, which found that low polymer concentrations were able to develop highly porous microspheres at room temperature. Briefly, zeta potential measures the surface charge of particles in samples. As the zeta potential values are larger than ±30 mV, the sample suspension is considered stable. This happens because the particles are pushing against each other more strongly, creating a dispersion suspension state. On the other hand, if the zeta potential value is less than ±30 mV, the particles are pulling against each other more strongly, creating an unstable suspension state that causes an aggregate to settle from the surrounding medium [29]. The value of zeta potential determines the stability of the microsphere. A higher absolute value of zeta potential could lead to better microsphere stability. The average zeta potential value of the microspheres was -30.7 mV±4.895, indicating strong repulsion forces among particles that might prevent aggregate formation. The high zeta potential value was believed to be due to the optimal ratio of emulsifiers (Tween 80 and Span 8 at 5:1 v/v) and concentration added to the oil and aqueous phases. The microspheres also had a negative charge because of an ionized carboxyl group that landed on their surfaces. This group came from lactic acid or glycolic acid in the PLGA [30,31].

Conclusion

Gelatin/PLGA microspheres were successfully fabricated using (W/O/W) emulsification followed by solvent evaporation. An emulsifying agent (Tween 80: Span 80 5:1) and a stabilizing agent (1% PVA) were incorporated into the double emulsion to improve emulsion stability and prevent particle coalescence; thus, they were responsible for the small microparticle size and high zeta potential values. The results showed that the particles were spread out and had a wide range of sizes. However, 23.53% of the microspheres were between 1 and 300 µm, which was good for incorporating cells and allowing injectable gauge passage.

Conflict of interests

No conflict of interests was declared by the authors.

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The project was financially supported by the Universiti Kebangsaan Malaysia (UKM) to purchase the materials used in the study. Additionally, the Hospital Universiti Kebangsaan Malaysia (HUKM) provided the gelatin required to conduct the experiments

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Yao Q, Wu X, Tao C, Gong W, Chen M, Qu M, et al. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther*. 2023;8:56. doi: 10.1038/s41392-023-01330-w.
2. da Costa BR, Reichenbach S, Keller N, Nartey L, Wandel S, Jüni P, et al. Effectiveness of non-steroidal anti-inflammatory drugs for the treatment of pain in knee and hip osteoarthritis: a network meta-analysis. *Lancet*. 2017;390(10090):e21–33. doi: 10.1016/S0140-6736(17)31744-0.
3. Ayhan E, Kesmezacar H, Akgun I. Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. *World J Orthop*. 2014;5(3):351–361. doi: 10.5312/wjo.v5.i3.351.
4. Montoya F, Martínez F, García-Robles M, Balmaceda-Aguilera C, Koch X, Rodríguez F, et al. Clinical and experimental approaches to knee cartilage lesion repair and mesenchymal stem cell chondrocyte differentiation. *Biol Res*. 2013;46(4):441–451. doi: 10.4067/S0716-97602013000400015.
5. Parel PM, Manyak GA, Carvajal JA, Abraham T, Al Rashid M. An early-stage comparison of functional outcomes following robotic-assisted versus conventional total knee arthroplasty: a systematic review and meta-analysis. *J Arthrosc Jt Surg*. 2022;9(3):77. doi: 10.4103/jajs.jajs_75_22.
6. Zhang L, Hu J, Athanasiou KA. The role of tissue engineering in articular cartilage repair and regeneration. *Crit Rev Biomed Engneer*. 2009;37(1–2):1–57. doi: 10.1615/critrevbiomedeng.v37.i1-2.10.
7. Solouk A, Mirzadeh H, Amanpour S. Injectable scaffold as minimally invasive technique for cartilage tissue engineering: in vitro and in vivo preliminary study. *Prog Biomater*. 2014;3:143–151. doi: 10.1007/s40204-014-0031-x.
8. Zhang Z. Injectable biomaterials for stem cell delivery and tissue regeneration. *Expert Opin Biol Ther*. 2017;17(1):49–62. doi: 10.1080/14712598.2017.1256389.
9. Kadam NR. Microsphere: a brief review. *Asian J Biomed Pharm Sci*. 2015;5(47):13-19. doi: 10.15272/ajbps.v5i47.713.
10. Malda J, van Blitterswijk CA, Grojec M, Martens DE, Tramper J, Riesle J. Expansion of bovine chondrocytes on microcarriers enhances redifferentiation. *Tissue Eng*. 2003;9(5):939–948. doi: 10.1089/107632703322495583.
11. Hossain KMZ, Patel U, Ahmed I. Development of microspheres for biomedical applications: a review. *Prog Biomater*. 2015;4(1):1–19. doi: 10.1007/s40204-014-0033-8.
12. Zhao C, Zhu Z, Cao X, Pan F, Li F, Xue M, et al. Evaluation the injectability of injectable microparticle delivery systems on the basis of injection force and discharged rate. *Eur J Pharm Biopharm*. 2023;190:58–72. doi: 10.1016/j.ejpb.2023.06.017.
13. Elzoghby AO. Gelatin-based nanoparticles as drug and gene delivery systems: reviewing three decades of research. *J Control Release*. 2013;172(3):1075–1091. doi: 10.1016/j.jconrel.2013.09.019.
14. Makadia HK, Siegel SJ. Poly cactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers (Basel)*. 2011;3(3):1377–1397. doi: 10.3390/polym3031377.
15. Yang H, Yao L, Wang Y, Chen G, Chen H. Advancing cell surface modification in mammalian cells with synthetic molecules. *Chem Sci*. 2023;14(46):13325–13345. doi: 10.1039/D3SC04597H.
16. Vedadghavami A, He T, Zhang C, Amiji SM, Hakim B, Bajpayee AG. Charge-based drug delivery to cartilage: Hydrophobic and not electrostatic interactions are the dominant cause of competitive binding of cationic carriers in synovial fluid. *Acta Biomater*. 2022;151:278–289. doi: 10.1016/j.actbio.2022.08.010.
17. Tsung MJ, Burgess DJ. Preparation and characterization of gelatin surface modified PLGA microspheres. *AAPS PharmSci*. 2001;3(2):E11. doi: 10.1208/ps030211.
18. Yasuda T. Cartilage destruction by matrix degradation products. *Mod Rheumatol*. 2006;16(4):197–205. doi: 10.1007/s10165-006-0490-6.

19. Chun KW, Yoo HS, Yoon JJ, Park TG. Biodegradable PLGA microcarriers for injectable delivery of chondrocytes: effect of surface modification on cell attachment and function. *Biotechnol Prog*. 2004;20(6):1797–1801. doi: 10.1021/bp0496981.
20. Mehrasa M, Asadollahi MA, Ghaedi K, Salehi H, Arpanaei A. Electrospun aligned PLGA and PLGA/gelatin nanofibers embedded with silica nanoparticles for tissue engineering. *Int J Biol Macromol*. 2015;79:687–695. doi: 10.1016/j.ijbiomac.2015.05.050.
21. Banerjee I, Mishra D, Maiti TK. PLGA microspheres incorporated gelatin scaffold: microspheres modulate scaffold properties. *Int J Biomater*. 2009;2009:143659. doi: 10.1155/2009/143659.
22. Tan H, Huang D, Lao L, Gao C. RGD modified PLGA/gelatin microspheres as microcarriers for chondrocyte delivery. *J Biomed Mater Res B Appl Biomater*. 2009;91(1):228–238. doi: 10.1002/jbm.b.31394.
23. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomater*. 2000;21(23):2475–2490. doi: 10.1016/S0142-9612(00)00115-0.
24. Morhenn VB, Lemperle G, Gallo RL. Phagocytosis of different particulate dermal filler substances by human macrophages and skin cells. *Dermatol Surg*. 2002;28(6):484–490. doi: 10.1046/j.1524-4725.2002.01273.x.
25. Zilberman M, Grinberg O. HRP-loaded bioresorbable microspheres: effect of copolymer composition and molecular weight on microstructure and release profile. *J Biomater Appl*. 2008; 22(5):391-407. doi: 10.1177/0885328207077591.
26. Choudhury M, Mohanty S, Nayak S. Effect of different solvents in solvent casting of porous pla scaffolds—in biomedical and tissue engineering applications. *J Tissue Sci Eng*. 2015;5(1):1–9. doi: 10.4172/2157-7552.1000142.
27. Pandya N, Pandya M, Bhaskar VH. Preparation and in vitro characterizatin of porous carrier–based glipizide floating microspheres for gastric delivery. *J Young Pharm*. 2011;3(2):97–104. doi: 10.4103/0975-1483.80292.
28. Ghanbar H, Luo CJ, Bakhshi P, Day R, Edirisinghe M. Preparation of porous microsphere-scaffolds by electrohydrodynamic forming and thermally induced phase separation. *Mater Sci Eng C*. 2013;33(5):2488–2498. doi: 10.1016/j.msec.2012.12.098.
29. Manaia EB, Abuçafy MP, Chiari-Andréo BG, Silva BL, Oshiro Junior JA, Chiavacci LA. Physicochemical characterization of drug nanocarriers. *Int J Nanomed*. 2017;12:4991–5011. doi: 10.2147/IJN.S133832.
30. Ruan G, Feng SS. Preparation and characterization of poly (lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomater*. 2003;24(27):5037–5044. doi: 10.1016/s0142-9612(03)00419-8.
31. Guo C, Gemeinhart RA. Understanding the adsorption mechanism of chitosan onto poly(lactide-co-glycolide) particles. *Eur J Pharm Biopharm*. 2008;70(2):597–604. doi: 10.1016/j.ejpb.2008.06.008.