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Tuning the Tensile Strength of Electrospun Fibers by Mesoporous Silica Nanoparticle Integration for Tissue Engineering Applications

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***Abstract*— The field of nanomedicine and tissue engineering has been gaining more and more interest in recent years. The use of nanoparticles in tissue engineering has brought a new dimension to this field. However, the mechanical features of scaffolds are the main factor for the governance of their potential applications. In this study, mesoporous silica nanoparticles (MSN) loaded electrospun PLGA scaffolds were manufactured. The morphology of MSN, PLGA fibers and PLGA-MSN integrated fibers were analyzed by electron microscopy imaging. The dispersity of MSN and zeta potential in colloidal suspension was analyzed. Subsequently in order to have understanding the mechanical properties of MSN loaded scaffolds; mechanical tests were performed. Results showed that MSNs are promising candidates in order to modify mechanical properties of the scaffolds and could be used as key components in various tissue engineering applications.**

Keywords — MSNs, PLGA, electrospinning, mechanical test.

I. INTRODUCTION

The field of tissue engineering relies on the use of porous three-dimensional (3D) scaffolds to ensure a suitable environment for the regeneration of tissues and organs. These scaffolds act as a template in order to restore, maintain or improve tissue function. Recent studies also have shown that mechanical properties of the scaffolds affect the behavior of cells, and guide proliferation or differentiation. Mechanical properties of the scaffolds can be enhanced by various approaches, such as by increasing crosslinking density. However, the toxic properties of the cross-linking agents can lead to decreased cell viability and other undesired effects. Therefore, new trends regarding the use of nanotechnology along with tissue engineering have emerged in recent years. Recent studies suggested that nanoparticles could be used to alter properties of the scaffolds.

Among the designed nanoparticles, mesoporous silica nanoparticles (MSN) provide great advantages due to their structural properties. MSN have shown to be a promising candidate in tissue engineering because of their numerous advantages, including biocompatibility, high loading capacity, controllable pore, and particle size. To date, MSNs have been mostly used with different polymers to enhance the mechanical properties of the scaffolds. Besides improved mechanical properties, the addition of MSNs allows improved cell adhesion, proliferation and even differentiation of cell lineages. Therefore, MSNs are of great importance in order to modify the physical characteristics of the scaffolds by only changing chemical properties and concentration of the MSNs [1].

The objective of this study is to investigate the effect of two different MSN types on PLGA scaffolds. The effect of spherical MSN (sMSN) and rod MSN (rMSN) over the mechanical properties of electrospun fiber PLGA scaffolds were examined with tensile tests. Effect of different percentages and morphology of MSNs over the properties the scaffolds were also studied. As a result, the utilization of MSNs in order to develop scaffolds with different characteristics was investigated in this feasibility study. According to obtained data, future studies will be carried out for *in vitro* evaluation of the developed scaffolds.

II. METHODS

A. Synthesis and Characterization of MSNs

1) MSN Synthesis

Synthesis of bare MSN was performed by using the sol-gel method. Tetraethyl orthosilicate (TEOS) was used as the silica source in the synthesis and cetyl-trimethyl-ammonium-bromide (CTAB) was structure directing agent. The synthesis was performed under basic condition in aqueous solution. After overnight reaction the products were collected with centrifugation and CTAB was removed by solvent extraction. After completely removing CTAB, the cake was suspended in an amount of ethanol and dispersed by sonication. Synthesized nanoparticles were stored at 4°C until further use. Henceforth the sample was named as sMSN.

Rod shaped MSNs (rMSN) were prepared according to SBA-15 type mesoporous material synthesis protocols as described in our previous study [2]. Briefly P123 (triblock

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copolymer) was used as structure directing agent and NH_4F were mixed in acidic aqueous reaction solution. Premixed TEOS and heptane added to reaction solution. The solutions were stirred for 4 min and then kept under static conditions at 20 ºC for 1 h and subsequently hydrothermal treatment at 100 ºC for 24 h. The products were filtered. Finally calcined at 550 ºC for 5 h to remove structure directing agent.

2) Zeta Potential and Hydrodynamic Size Measurements

The hydrodynamic size and Zeta potential of the synthesized MSNs was measured by dispersing MSNs in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma Aldrich) buffer solution (pH=7.2, 25 mM).

3) Scanning Electron Microscopy

SEM analysis (Carl Zeiss 300VP) of MSNs and PLGA scaffold was performed in order to investigate the morphology of the synthesized materials. Approximately 100 µl of dispersed MSN solutions were spread onto glass coupons evenly and allowed to dry in a dust-free environment. On the following day, imaging was performed after sputtering all the samples with gold.

B. Preperation and Characterization of MSN-loaded Scaffolds

1) Preperation of pristine PLGA electrospun fibres

In order to perform electrospinning of PLGA (Purasorb® PDLG 5010), PLGA pellet (7% (w/v)) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP; Matrix Scientific) for 30 min using a magnetic stirrer. The solution was then electrospun (NanoWEB 103) using a 5 ml syringe with a blunt-end needle and a mass flow rate of 1ml/h. The distance between the needle tip and the metallic collector was 15 cm while the applied voltage was chosen to be 20 kV. Fabricated nanofibers were then stored at room temperature for further analyses.

SEM analysis for samples was carried out by using PLGA samples having a size of 1 cm x 1cm. Samples on carbon tape were sputtered with gold before analysis.

2) Incorporation of MSNs into Scaffolds

Three different concentrations of both rod and spherical MSNs were incorporated into scaffolds. For the MSN-loaded PLGA scaffolds, firstly MSNs (1, 5 and 10 wt% total weight of PLGA) were mixed with 250µl milli-Q water and sonicated for 20 min. Sonicated MSNs were then added to the dissolved PLGA solution drop wisely. The resulting mixture was sonicated and stirred for 30 min to homogeneously disperse the MSNs in the polymer solution. Fibers were then fabricated under the same conditions mentioned in Section B.1.

3) Tensile Test

MSN-loaded PLGA samples were prepared according to determined size and subjected to a tensile test with the loading rate of 10 mm/min (Shimadzu AG-IC). Stress vs elongation curve was obtained for the samples containing different amounts of MSNs according to obtained elongation ratios. Three replicates of each sample were used for analysis.

III. Results And Discussion

A. Characterization of MSNs

The morphology and structural characteristics of MSNs were analyzed by different techniques like SEM, and Zeta potential measurements, as shown in Table I and Fig. 1.

The SEM micrographs of rMSNs and sMSNs demonstrate particle size of ~200 nm. Table I shows the polydispersity index (PDI) of the synthesized nanoparticles supporting their colloidal stability. Zeta potential values of the nanoparticles showed the net negative surface charged MSNs.

TABLE I. ZETA POTENTIAL AND POLYDISPERSITY INDEX VALUES OF PRISTINE SMSN/RMSN

Type	*Zeta Potential (mV)*	*Polydispersity Index*
sMSN	-5.08±0.74	0.84
rMSN	-19±0.1	0.12

B. Characterization of MSN-loaded PLGA electrospun fibers Scaffolds

The SEM images of electrospun PLGA nanofibers are shown in Fig. 1. Working conditions of electrospinning process were previously optimized for the concentration of polymer, distance, applied voltage, and flow rate. The pure PLGA had a fiber diameter between 210-420 nm, Fig. 1 also showed that the fabricated nanofibers were smooth, continuous and bead-free. Chen et al. obtained thinner fibers when MSN was incorporated into poly (methyl methacrylate) solution [3]. Seemingly, the conductivity of the polymer solution increases in the presence of MSNs because composite fibers having lower diameters were fabricated.

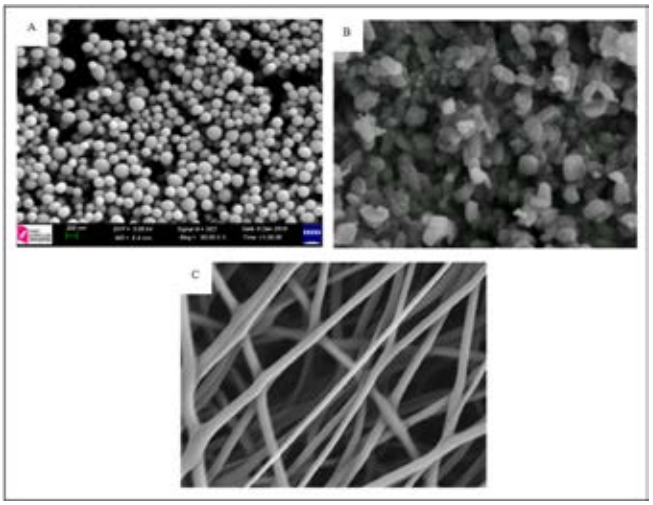

Fig. 1. SEM micrographs showing A) sMSNs, B) rMSNs, C) PLGA scaffolds.

Stress vs. % elongation graph is shown in Fig. 2 for sMSN and rMSN-loaded PLGA fibers. sMSN-incorporated PLGA

nanofibers have increased ultimate tensile strengths (UTS) compared to pristine PLGA group for all concentrations of sMSNs which are 1%, 5%, and 10%. As in sMSNs-loaded PLGA fibers, rMSNs-loaded PLGA fibers also display increased tensile strength. However, for rMSNs, there is an inverse relationship between UTS and nanoparticle concentration. with respect to increased rMSN concentration. In addition, presence of sMSNs and rMSNs in PLGA scaffolds increased hardness of the samples compared to pristine PLGA group.

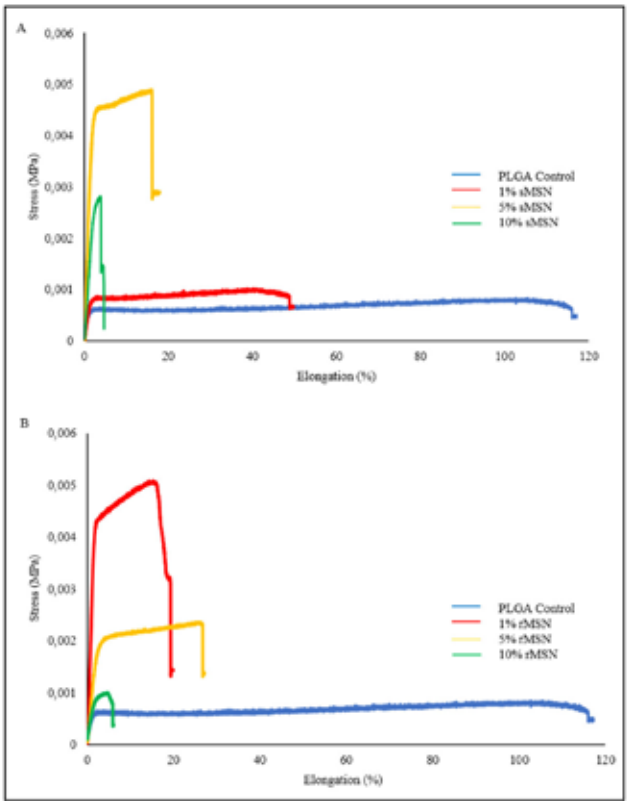

Fig. 2. Stress vs Elongation graphs for A) sMSN-loaded PLGA scaffolds, B) rMSN-loaded PLGA scaffolds

Effect of different MSNs concentration on the elastic modulus of PLGA fibers is also demonstrated in Fig. 3. Results showed that all concentrations of both sMSNs and rMSNs led

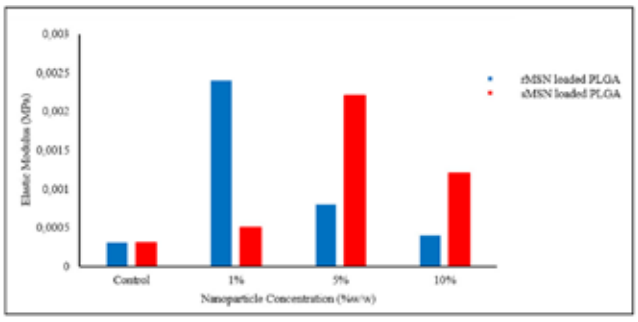

Fig. 3. Graph showing the relationship between elastic modulus vs. nanoparticle concentration for MSN-loaded PLGA scaffolds.

to an increase in elastic modulus of PLGA fibers to some extent. 1% rMSN-loaded PLGA scaffold had the highest elastic modulus indicating high elongation property. As rMSN concentration increases, a gradual decrease in the elongation property of the composite scaffolds was observed. For sMSN-loaded PLGA scaffolds, there is an increment in elastic modulus up to concentration of 5%. Yet, at 10% of sMSN, there is a substantial decrease in elastic modulus. This result can be explained by possible agglomeration of the sMSNs during electrospinning process.

Apart from agglomeration of MSNs in samples, alignment of the PLGA fibers in samples, alignment and localization of the nanoparticles in scaffolds and variability in the thickness of the fiber mats may have high impact on the elastic modulus and tensile strength.

Yang and co-workers also used silica nanoparticles in order to enhance the mechanical behavior of PLGA fibers. Additionally, the use of silica nanoparticles increase attachment and spreading of osteoblast-like cells (SaOS-2 cells) on composite fibers. With increasing concentrations of silica nanoparticles in PLGA fibers, it was also observed that bone nodules formation of SaOS-2 cells showed an increase. They showed that presence of silica nanoparticles results in an increment in hardness and elastic modulus of PLGA fibers. PLGA fiber having the highest amount of silica nanoparticles (5%) had the highest elastic modulus. These results supported the reinforcing effect of silica nanoparticles on the PLGA matrix [4].

Mehrasa et al also studied the effect of silica nanoparticles over the mechanical properties of the PLGA and PLGA/gelatin fibers. According to results, increased tensile strength and Young's modulus were obtained with the addition of MSNs into the fibers. Increased Young's modulus has been explained by the restricted mobility of the PLGA molecules because of the dispersed MSNs in the composite matrix [5], [6].

IV. Conclusion

In this study, sMSN and rMSN-loaded PLGA scaffolds were successfully fabricated and investigated in terms of their mechanical properties. Tensile tests revealed that incorporation of both types of rod and spherical shaped MSNs resulted in improved elastic modulus along with increased tensile strengths. These results clearly indicate that composite scaffolds comprising nanoparticles exhibit tunable mechanical behavior which could help also to improve cell attachment and differentiation for use in different tissue engineering applications in the future.

References

[1] X. Zhou *et al.*, "Mesoporous silica nanoparticles/gelatin porous composite scaffolds with localized and sustained release of vancomycin for treatment of infected bone defects," *J. Mater. Chem. B*, vol. 6, no. 5, pp. 740–752, 2018.
[2] D. Sen Karaman *et al.*, "Shape engineering vs organic modification of inorganic nanoparticles as a tool for enhancing cellular internalization," *Nanoscale Res. Lett.*, vol. 7, no. July, pp. 2–32, 2012.
[3] Y. Z. Chen, Z. P. Zhang, J. Yu, and Z. X. Guo, "Poly(methyl methacrylate)/silica nanocomposite fibers by electrospinning," *J.*

Polym. Sci. Part B Polym. Phys., vol. 47, no. 12, pp. 1211–1218, Jun. 2009.
[4] X. Yang, Y. Li, X. Liu, Q. Huang, R. Zhang, and Q. Feng, "Incorporation of silica nanoparticles to PLGA electrospun fibers for osteogenic differentiation of human osteoblast-like cells," no. June, pp. 229–238, 2018.
[5] M. Mehrasa, M. Ali, K. Ghaedi, H. Salehi, and A. Arpanaei, "International Journal of Biological Macromolecules Electrospun aligned PLGA and PLGA / gelatin nanofibers embedded with silica nanoparticles for tissue engineering," *Int. J. Biol. Macromol.*, vol. 79, pp. 687–695, 2015.
[6] M. Mehrasa *et al.*, "Incorporation of mesoporous silica nanoparticles into random electrospun PLGA and PLGA/gelatin nanofibrous scaffolds enhances mechanical and cell proliferation properties," *Mater. Sci. Eng. C*, 2016.