PRODUCTION AND CHARACTERIZATION OF HYALURONIC ACID NANOFIBERS CONTAINING TGF-β AND BAICALEIN BY USING ELECTROSPINNING METHOD

ELEKTROSPIN YÖNTEMIYLE BAICALEIN VE TGF-β IÇEREN HYALURONIK ASIT NANOFIBERLERININ ÜRETIMI VE KARAKTERIZASYONU

KAMEL BACHIMAM

PROF. DR. NECDET SAĞLAM
Supervisor

Submitted to Institute of Graduate School of Science and Engineering of Hacettepe University as a Partial Fulfillment to the Requirements for the Award of the Degree of Master Science in Nanotechnology and Nanomedicine

2017
This work named "Production and Characterization of Hyaluronic Acid Nanofibers containing TGF-β and Baicalein by Using Electrosprinning Method" by KAMEL BACHIMAM has been approved as a thesis for the degree of Master in Nanotechnology and Nanomedicine by the below mentioned Examining Committee members.

Prof. Dr. Uğur TAMER  
Head  

Prof. Dr. Necdet SAĞLAM  
Supervisor  

Prof. Dr. Feza KORKUSUZ  
member  

Doç. Dr. Hakan ATEŞ  
member  

Yrd. Doç. Dr. Mesut ŞAM  
member  

this thesis has been approved as a thesis for the degree of Master in Nanotechnology and Nanomedicine by Board of Directors of the institute for Graduate School of Science and Engineering  

Prof. Dr. Menemşe GÜMÜŞDERELİoğlu  
Director of the Institute of Graduate School of Science and Engineering
YAYINLAMA VE FIKRİ MÜLKİYET HAKLARI BEYANI

Enstitü tarafından onaylanan lisansüstü tezinin/raporun tamamını veya herhangi bir kısmını, basılı (kağıt) ve elektronik formatta arşivleme ve aşağıda verilen koşullara kullanılmaya açma iznini Hacettepe Üniversitesine verdim. Bu izinde Üniversiteye verilen kullanımlar dışındaki tüm fikri mülkiyet haklarının benim kalanak, tezinin tamamının ya da bir bölümüne gelecekteki çalışmalarında (makale, kitap, lisans ve patent vb.) kullanım haklarını bana ait olacaktır.

Tezin kendi orijinal çalışmanın olduğunu, başkalarının haklarını ihlal etmediğini ve tezinin tek yetkili sahibi olduğunu beyan ve taahhüt ederim. Tezinde yer alan telif hakkı bulunan ve sahiplerinden yazılı izin alınarak kullanılması zorunlu metinlerin yazılı izin aralarak kullanılması ve istendiğinde suretlerini Üniversiteye teslim etmeyi taahhüt ederim.

☐ Tezinin/Raporunun tamamı dünya çapında erişime açılabilir ve bir kısmı veya tamaminin fotokopisi alınabilir.
(Bu seçeneke tezinin arama motorlarında indekslenebilecek, daha sonra tezinizin erişim statüsünün değiştirilmesini talep etsiniz ve kutuphaneye bu talebinizi yerine getirirse bile, tezinin arama motorlarının önbelliklerinde kalmaya devam edebilecektir)

☐ Tezinin/Raporunun ___________ tarihine kadar erişime açılmasını ve fotokopi alınmasını (İç Kapak, Özeti, İçindekiler ve Kaynakça hariç) istemiyorum.
(Bu sürenin sonunda uzatma için başvuruda bulunmadığım taktirde, tezinin/raporun tamamı her yerden erişime açılabilir, kaynak gösterilmek şartıyla bir kısmı ve ya tamaminin fotokopisi alınabilir)

☐ Tezinin/Raporunun ___________ tarihine kadar erişime açılmasını istemiyorum, ancak kaynak gösterilmek şartıyla bir kısmı veya tamaminin fotokopisinin alınmasını onaylıyorum.

☐ Serbest Seçenek/Yazarın Seçimi

26 / 06 / 2017

(Lns)
To My Family
ETHICS

In this thesis study, prepared in accordance with the spelling rules of Institute of Graduate Studies in Science of Hacettepe University,

I declare that

- all the information and documents have been obtained in the base of academic rules
- all audio-visual and written information and results have been presented according to the rules of scientific ethics
- in case of using others Works, related studies have been cited in accordance with the scientific standards
- all cited studies have been fully referenced
- I did not do any distortion in the data set
- and any part of this thesis has not been presented as another thesis study at this or any other university.

14/06/2017

KAMEL BACHIMAM
ÖZET

ELEKTROSPİN YÖNTEMİYLE BAİCALEİN VE TGF-B İÇEREN HYALURONİK ASİT NANOFİBERLERİNİN ÜRETİMİ VE KARAKTERİZASYONU

KAMEL BACHIMAM
YÜKSEK LİSANS, NANOTEKNOLOJİ VE NANOTIP ANABİLİM DALI
TEZ DANIŞMANI: PROF. DR. NECDET SAĞLAM
HAZİRAN 2017, 98 SAYFA

Temelde, kemik dokusu kaybı iki sebepten kaynaklanmaktadır. İlk olarak kaza gibi acil bir durumdan ötürü gerçekleşirken, ikinci olarak ise kanser tedavisi operasyonu gibi (primer kemik kanseri yada kemiğe metastatik olan prostat kanseri) planlanmış bir şekilde gerçekleşmektedir. Doktorlar eksizyonel operasyon sonrası kanserli dokunun doku kaybı alanını dolduran ve aynı zamanda sadece kemik entegrasyonunun iyileştirilmesine izin vermeyen, kemik rekonstrüksiyonunu destekleyebilen ve hala mevcut olan kanser hücrelerinin yok edilmesini sağlayacak olup işlevsel bir yapı taşıyabilen eşsiz bir işlev ve özelliklere sahip biyolojik olarak uyumlu materyallerin ihtiyacı duymaktadırlar.

Son birkaç yılıdır bir çin bitkisel ilacı olan baicalein’in özellikle kanser hücrelerinin çoğunmasına ve hücre metastazına karşı bir inhibitör görevi üstlenmesi üzerinde araştırma ve çalışmalar çok sayıda yapılmaktadır. Söz konusu ilacin, genellikle
prostat, göğüs, lösemi gibi kanser uygulamalarına sıkça rastlanmaktadır fakat prostat kanseri kemik metastazı tedavisinin uygulaması fazla bulunmamaktadır.

Diğer taraftan baicalein'in elektrospinning yöntemiyle, kemik rejenerasyonu için çevresine antikanserojen moleküllerini salabilen bir sentetik iskelet olarak kullanılması; yüksek biyouyumluluk derecesine ve diğer antikanserojenlere nazaran düşük toksisiteye sahip olması nedeniyle olası görülmektedir.

Çalışmamızda, iki farklı polimer çözeltisinden nanofiber esaslı iskelet yapısı üretmek için Elecspinning yöntemi kullanıldı; elektrospinning çözeltileri Hyaluronik asit, polietilen oksit ve TGF-beta 2, medyumu içerenken ayrıca farklı konsantrasyonda Baicelein molekülleri ile karıştırılmış polivinil alkol içerir. Oluşturulan nanofiber esaslı iskelet yapısının karakterizasyon değerlendirmesi yapmak için Taramalı elektron mikroskopu (SEM) görüntüleri elde edilmiş ve ayrıca iskelet örneklerinin kimyasal bileşimini incelemek için Fourier Dönüşümü Kıızılojesi - Spektroskop Analizi testi (FT-IR) yapılmıştır.

Anahtar kelimeler: Baicalein, Prostat Kanseri, Kemik Kanseri, Elektrospinning – Nanofiber, Hyaluronic Asit – TGF-Beta2
Bone Tissue loss considered as one of the frequent cases that require a surgical intervention. whether the case needs an urgent treatment as in huge accidents, or performed in terms of preplanned operation as in bone cancer treatment or palliative surgical intervention in case of prostatistic metastatic cancer invasion in bone tissue, orthopedics still need biologically compatible materials that can be used to fill the space of tissue loss, and in the same time posses a unique function and properties that not just allow improve the bone integration, but also it can carry a functional elements that can support bone reconstruction and ensure destruction of all cancer cells remnants still present in the tissue after excisional operation to the cancer bulk tissue.

In the last few years an increasing number of papers discussing the ability to
produce a synthetic scaffold used in bone transplantation that can release anticancer molecules with a high biocompatibility level with the surrounding tissues. For this purpose, Electrospinning method has attracted the attention of scientists since it provides a simple, quick and cheap way to produce nanofiber-based scaffold that mimic the natural structure of human body extracellular matrix and can serve as a drug delivery system that can release anticancer molecules with a high biocompatibility level with the surrounding tissues.

In the same time, we can find a big number of studies carried out on Baicalein substance aiming to understand the various outstanding properties that such a herbal Chinese medicine can possess, particularly for treatment of a wide range of diseases especially its inhibitory effects on cancer cells proliferation and cell metastasis.

In our study, we used Electrospinning method to produce nanofiber based scaffold structure from different polymer solutions; Hyaluronic acid blended with Polyethylene oxide med with TGF-beta 2 and also polyvinyl alcohol mixed with different concentration of Baicalein molecules. We perform characterization assessment to the produced nanofiber based scaffold sample by getting scanning electron microscope (SEM) images and also doing Fourier Transform Infrared - Spectroscopy Analysis test (FT-IR) to study the chemical composition of the scaffold samples.

Key wards: Baicalein, bone cancer, Metastatic prostate cancer, Electrospinning nanofiber, Hyaluronic acid, TGF-β 2
THANKS

By the End of this work I want to thank My Father and Mother who are still living in my country, wide from Ankara, yet they didn’t miss any chance to let me feel that I’m not alone, and although we are staying in different places, but still they can give the needed support that all people seek from their parents. Also My Sister was insisting on me all the time to ask her to explain any thing for me or even help me in the writing of these thesis, and I was really happy to get benefit from her wide academic experience. My brother has also support me her in Ankara by offering all type of helps for me.

Also I have My work Family at Hacettepe University, and the head of it Prof. Dr. Necdet SAĞLAM, who was, as I felt during 3 years of work, not just a respected Professor who just give the lesson or give the explain the way of work in Laboratory, but he was really trying to make more deep relation with all his students, supports them, helps them, solve their problems, and even shares his feelings in both happiness and sadness. I think he was in a position of Father for all his students. The second effective member of my academic family was PhD student Ezgi Emül, who also has offered me a huge ammount of support, She helped me during my Lab works steps, told me about the samll details that I caould miss them, and give lot of her time to ensure that all my document and forms needed for Institute are all complete with no missing of any information for the last minute of this Thesis.

İn the ame context, I wou;d be happay to thank the committee member of my Thesis starting with the Committee Head; Prof,Dr. Uğur TAMER, Prof,Dr. Feza KORKUSUZ, Doç. Dr. Hakan ATEŞ, Yrd. Doç. Mesut ŞAM, to whom I really appreciate their discussion and understanding during my thesis presentation. Moreover, I really want to thank Doç. Dr. Memed DUMAN and his Laboratory staff who let me work in the Laboratory with a high level of comfortable and huge amount of laboratory work support, always with a smile faces and respectful attitude.
Finally, I want to thank Hacettepe University which gave me this precious chance to get Master degree in Nanotechnology and Nanomedicine Sciences and gaining a big amount of academic experiences that will support me during my future life.

My thesis was funded by Hacettepe BAP (Bilimsel Araştırma Projeleri Kordinasyon Birimi) for funding of post graduate students projects (project ID: 9112).
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÖZET</td>
<td>III</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>V</td>
</tr>
<tr>
<td>THANKS</td>
<td>VII</td>
</tr>
<tr>
<td>TABLE OF FIGURES</td>
<td>XI</td>
</tr>
<tr>
<td>TABLE OF TABLES</td>
<td>XV</td>
</tr>
<tr>
<td>UNITS VE ABREVIASION</td>
<td>XVI</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. GENERAL INFORMATION</td>
<td>8</td>
</tr>
<tr>
<td>2.1. ELECTROSPINNING AND NANOFIBER SCAFFOLDS</td>
<td>8</td>
</tr>
<tr>
<td>2.2. ELECTROSPINNING</td>
<td>9</td>
</tr>
<tr>
<td>2.2.1. HISTORY OF ELECTROSPINNING</td>
<td>9</td>
</tr>
<tr>
<td>2.2.2. APPARATUS AND METHOD</td>
<td>10</td>
</tr>
<tr>
<td>2.2.3. IMPORTANCE OF PROCESS’S PARAMETERS</td>
<td>12</td>
</tr>
<tr>
<td>2.2.4. NANOFIBERS’ COMPONENT MATERIALS</td>
<td>15</td>
</tr>
<tr>
<td>2.2.4.1. HYALURONIC ACID</td>
<td>15</td>
</tr>
<tr>
<td>2.2.4.1.1. APPLICATION OF HA</td>
<td>19</td>
</tr>
<tr>
<td>2.2.4.1.2. HA BASED SCAFFOLD APPLICATION</td>
<td>21</td>
</tr>
<tr>
<td>2.2.4.2. POLYETHYLENE OXIDE</td>
<td>22</td>
</tr>
<tr>
<td>2.2.5. APPLICATION OF NANOFIBER FORMATION</td>
<td>23</td>
</tr>
<tr>
<td>2.2.5.1. LITHIUM-AIR BATTERY</td>
<td>23</td>
</tr>
<tr>
<td>2.2.5.2. FILTRATION</td>
<td>24</td>
</tr>
<tr>
<td>2.2.5.3. COATING AND PACKAGING</td>
<td>25</td>
</tr>
<tr>
<td>2.2.5.4. CANCER DIAGNOSIS</td>
<td>27</td>
</tr>
<tr>
<td>2.2.5.5. TISSUE ENGINEERING</td>
<td>29</td>
</tr>
<tr>
<td>2.2.5.5.1. TISSUE GRAFTING</td>
<td>30</td>
</tr>
<tr>
<td>2.2.5.5.2. DRUG DELIVERY SYSTEMS</td>
<td>34</td>
</tr>
<tr>
<td>2.2.5.5.2.1. BAICALEIN</td>
<td>37</td>
</tr>
</tbody>
</table>
# TABLE OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Percentage of Malignant Bone Cancer</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Comparison between (A) Normal Tissue Histological Structure and (B) Nanofibers Based Scaffold Structure</td>
<td>6</td>
</tr>
<tr>
<td>2.1</td>
<td>Electrospinning Setup</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>Calculated Final Jet Cross Sectional Radius by the Changing in the Polymer Concentration</td>
<td>14</td>
</tr>
<tr>
<td>2.3</td>
<td>Fiber spun from different concentration; (A) 8%wt solution resulted in fiber of 300 Nm and (B) 12%wt solution resulted in fiber with 750 Nm in diameter (From D. Hussain, PhD Thesis, Department of Chemistry, Philips Universitat) (Marburg 2009)</td>
<td>14</td>
</tr>
<tr>
<td>2.4</td>
<td>The relation between spun fiber diameter and polymer concentration; Using Polyacrylonitrile (Pan) dissolved in N-Dimethylformamide (From D. Hussain, PhD Thesis, Department of Chemistry, and Philips Universitat) (Marburg 2009)</td>
<td>15</td>
</tr>
<tr>
<td>2.5</td>
<td>Hyaluronic Acid</td>
<td>16</td>
</tr>
<tr>
<td>2.6</td>
<td>The structural position of Ha inside ECM; Inset displays a higher magnification of the Aggrecan molecule, indicating the core protein of the proteoglycan molecule to which the glycosaminoglycans are attached. The core protein is attached to the hyaluronic acid by link proteins. (Adapted from Fawcett Dw: Bloom and Fawcett’s A Textbook of Histology, 11th Ed. Philadelphia, Wb, Saunders, 1986.)</td>
<td>19</td>
</tr>
<tr>
<td>2.7</td>
<td>Polyethylene Oxide</td>
<td>23</td>
</tr>
<tr>
<td>2.8</td>
<td>Snf/Fca filtration membrane; the membrane demonstrate a NOil-Water separation properties (After 30 Seconded the E Water Remain Upside, while the oil crossed the membrane to fall down the bahar.[88]</td>
<td>25</td>
</tr>
<tr>
<td>2.9</td>
<td>Using Polyurethane / Polyethylene Nanofiber Film for Breathable Waterproof Fabric Formatim [90]</td>
<td>26</td>
</tr>
<tr>
<td>2.10</td>
<td>Cross-Section Image of Multilayer Prepared Tipc-15%Bcnw As Inner</td>
<td></td>
</tr>
</tbody>
</table>
Layer Nd Phb-Bcnw As Outer Layers ................................................................. 27
Figure 2.11. Schematic Representation Of The Fabrication Of Carcinoma Antigen-125 Immunosensor [94] ................................................................. 28
Figure 2.12. Nanovelcro Chip For Circulating Tumor Cell (Ctc) Detection .......... 29
Figure 2.13. Creating Multi-Layered Scaffolds From Electrospun And Non- Electrospun Mats And The Architecture Of The Prototype Layered Electrospun/Woven Scaffold; The Left Figure Shows The Structural Positions Of Pdo And Pcl Nanofiber-Layers [105] ......................................................... 31
Figure 2.14. 3d Scaffold Of Aortic Heart Valve Including Leaflets And Root ...... 32
Figure 2.15. Fe-Sem Images Of Cell Growth As A Function Of Incubation Time (Days) ........................................................................................................ 33
Figure 2.16. Representative Confocal Laser Scanning Microscope Images Of Pc 12 Cells Cultured For 3 Days On (A) L-Prpn Composite Micro/Nano-Fibrous Scaffold, (B) On Control [111] .......................................................... 34
Figure 2.17. Re-Growth Of Neural Tissue After L-Prpn Composite Transplantation ................................................................. 34
Figure 2.18. Co-Axial Fiber Electrospinning; (A) Device Setup , (B) Sem Image Of Core/Shell Nanofiber [115] ........................................................................ 36
Figure 2.19. Baiacelin Herbs And Its Roots ........................................................................ 38
Figure 2.20. Baiacelin 5,6,7-Trihydroxyflavone 5, 6, 7-Trihydroxy-2-Phenyl-(4h)-1- Benzopyran-4-One” ........................................................................ 38
Figure 2.21. Mechanism Of Baiacelin For Amelioration Of Different Inflammatory Diseases .................................................................................. 40
Figure 2.22. Electrospinning Device ........................................................................ 49
Figure 4.1. Our Produced Peo/Ha/Tgf-Beta 2 + Pva/Baicalein Nanofiber Scaffold Samples With Different Baiacelin Concentration(A1, A2) 0.2% Baiacelin, (B1, B2) 0.5% Baiacelin, (C1, C2) 1% Baiacelin, (D1, D2) 2% Baiacelin. ......................... 52
Figure 4.2. Sem Images Of Electrospun Peo/Ha 5%Wt Nanofibers; (A) Peo/Ha 10/1, (B) Peo/Ha 5/1 Show High Level Of Beads Formation................................. 56
Figure 4.3. Sem Image Of Electrospun Peo/Ha (5%/ 2%) Nanofibers ; (A) 10.000 X Magnification, (B) 40.000 X Magnification Show Medium Level Of Beads Formation ........................................................................ 57
Figure 4.4. Sem Image Of Electrospun Peo/Ha (10% / 0.1%) Nanofibers (A) 10.000 X Magnification , (B) 10.000 X Magnification And (C) 40.000 Magnification Show
Disappearing Of Beads Within The Produced Nanofibers................................. 58
Figure 4.5. Sem Image Of Electrospun Pva Nanofibers In 50.000x (A) 13%Wt Pva , (B) 10%Wt Pva And (C) 7%Wt Pva Show Different Nanofiber Diameters.............. 59
Figure 4.6. Sem Image Of Electrospun Peo/Ha/Tgf-Beta2 With Pva / 0% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.............................. 60
Figure 4.7. Sem Image Of Electrospin Peo/Ha/Tgf-Beta2 With Pva / 0.2% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer. Also The Three Image Clearly Show Degradation Happened To The Nanofibers After 3 Weeks Of Production................................................................. 61
Figure 4.8. Sem Image Of Electrospun Peo/Ha/Tgf-Beta2 With Pva / 0.5% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.............................. 62
Figure 4.9. Sem Image Of Electrospun Peo/Ha/Tgf-Beta2 With Pva / 1% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.............................. 63
Figure 4.10. Sem Image Of Electrospun Peo/Ha/Tgf-Beta2 With Pva / 2% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.............................. 64
Figure 4.11. Distribution Of Nanofiber Diameters Measurements For Peo/Ha/Tgf-Beta2 With Pva / 0% Baicalein Nanofibers................................................................. 65
Figure 4.12. Distribution Of Nanofiber Diameters Measurements For Peo/Ha/Tgf-Beta2 With Pva / 0.2% Baicalein Nanofibers................................................................. 65
Figure 4.13. Distribution Of Nanofiber Diameters Measurements For Peo/Ha/Tgf-Beta2 With Pva / 0.5% Baicalein Nanofibers................................................................. 66
Figure 4.14. Distribution Of Nanofiber Diameters Measurements For Peo/Ha/Tgf-Beta2 With Pva / 1% Baicalein Nanofibers................................................................. 67
Figure 4.15. Distribution Of Nanofiber Diameters Measurements For Peo/Ha/Tgf-Beta2 With Pva / 2% Baicalein Nanofibers................................................................. 67
Figure 4.16. Ft-Ir Spectrum For Peo, Ha And Pva.................................................. 69
Figure 4.17. Ft-Ir Spectrum For Baicalein ............................................................. 70
Figure 4.18. Ft-Ir Spectrum For Peo/Ha/Tgf-Beta2 (Above), And Pva/Baicalein (Below)......................................................................................................................... 71
Figure 4.19. Ft-Ir Sectrum For Peo/Ha/Tgf-Beta2 - Pva /0% Baicalein .......... 72
Figure 4.20. Ft-Ir Sectrum For Peo/Ha/Tgf-Beta2 - Pva /0.2% Baicalein ........ 74
Figure 4.21. Ft-Ir Sectrum For Peo/Ha/Tgf-Beta2 - Pva /0.5% Baicalein ........ 74
Figure 4.22. Ft-Ir Sectrum For Peo/Ha/Tgf-Beta2 - Pva /1% Baicalein .......... 75
Figure 4.23. Ft-Ir Sectrum For Peo/Ha/Tgf-Beta2 - Pva /2% Baicalein .......... 76
Figure 4.24. Ft-Ir Sectrum For Peo / Ha / Baicalein.................................. 77
# TABLE of TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Review Of Some Drug Pharmacokinetic Obstacles And How Dds Improve The Drug Efficiency</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Therapeutic Effects Of Baicalein On Some Inflammatory Diseases</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>Effects Of Baicalein On Number Of Cancer Diseases</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>Other Therapeutic Effects Of Baicalein On Various Diseases</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>Review 1 Of Different Polymer Solutions Spinning Findings That Used In Our Study, And The Sem Appearance Results For Each One Of Them</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>Review 2 Of Different Polymer Solutions Spinning Findings That Used In Our Study, And The Sem Appearance Results For Each One Of Them</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>Review 3 Of Different Polymer Solutions Spinning Findings That Used In Our Study, And The Sem Appearance Results For Each One Of Them</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>Review 4 Of Different Polymer Solutions Spinning Findings That Used In Our Study, And The Sem Appearance Results For Each One Of Them</td>
<td>54</td>
</tr>
</tbody>
</table>
# UNITS VE ABRÉVIASION

## Units

<table>
<thead>
<tr>
<th>Unit</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>wt</td>
<td>weight</td>
</tr>
</tbody>
</table>

## Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td>PEO</td>
<td>Polyethylene oxide</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>Bias</td>
<td>Baicalein</td>
</tr>
<tr>
<td>CA</td>
<td>Cellulose acetate</td>
</tr>
<tr>
<td>Conc.</td>
<td>Concentration</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic-acid)</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolacton</td>
</tr>
<tr>
<td>PPY</td>
<td>Polypyrrole</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Bone tissue loss considered one of the frequent cases that require surgical reconstruction interaction particularly in case of having a relatively big amount of bony tissue has been lost. In general, Orthopedic doctors used to do Reconstructive surgery to the bone tissue lesion in tow case; First, when the patient suffer from an acute bone damage which can be seen as one of the complicated bone fracture due to accident or being at the site of big explosion. Whereas the second case that can be called a pre-planned or therapeutic interventional type this kind of operation is widely done for different therapeutical purposes. The most popular indication is the presence of cancer tissue that needs, according its type and the stage, to perform a wide-exciscional surgical operation to remove the oncogenic tissue completely. In some cases the surgical operation will be combined with further radiotherapy and chemotherapy to ensure destruction of all cancer cell remnants that may cause re-growth to the tumor a gain in future.

Basically, bone cancer can be either primary cancer, which emerged from the bone itself, or secondary cancer that results from metastatic cancer cell movement from its origin site like Prostate, Breast and Liver to settle down and cause a new tumor growth in side a healthy bone tissue. Primary bone tumor can be found as benign bone tumor that has no risk to move and spread out to other body organs like:

- Osteoid osteoma
- Osteoblastoma
- Osteochondroma
- Enchondroma
- Chondromyxoid fibroma

These benign cancer can be treated surgically by removing them from the surrounding bone tissue, thus may result of tissue loss that may arrange between small and large loss according to the size of cancer growth inside the bone. Primary bone tumor also include the malignant type that has the liability to move
out and spread to other organs, therefore we can find different therapeutic methods that include in most cases combination of more than one method like applying of radio therapy and chemotherapy before or after the surgical treatment. This difference in the different therapeutic methods may depend on the type and degree of cancer malignancy. The most frequently diagnosed malignant bone cancer is chondrosarcoma (40%), and then comes osteosarcoma at about 31%. Than chondromas(11%) , Ewing tumors(9%) malignant fibrosarcomas have the least incidence rate at about 5%. In the same context, there are other types of cancer cells that emerge from the bone marrow. this kind of cancer has different developmental rout since it effect the blood cell compartments, thus has distinct therapeutic methods like Myelomas and Primary non-Hodgkin lymphomas.[1]

![Figure 1.1 Percenage of Malignant Bone Cancer](image)

However, tumor can sometimes move out its compartment and spread out via the blood stream to reach the bone tissue. This case called Secondary Bone Tumor and it can see frequently in the patient who suffers from malignant Prostate cancer, Breast cancer and Lund cancer.

In case of having prostate cancer metastasis to the bone, doctors conventionally apply on of next treatment options:

Androgen deprivation therapy (ADT) that affects the velocity of cancer growth.
Hormonal therapies.

Chemotherapy, often used after the body stops responding to hormone therapy

Vaccines and immunotherapy.

Radiotherapy.

Ablation technique, using a needle to destroy tumors with heat, cold, or electric currents[2].

Depending on the former fact and information, in both types of bone tumor whether its primary or secondary bone tumor, doctors might use the surgical therapeutical operation (either primary surgical treatment or as palliative destruction to the metastatic cancer sites) that may results in tissue loss to the bone compartment and require further reconstruction operation to fill the gap that happened in the bone tissue after removing of cancer. For this purpose, Orthopedists can use the one of the different types of Bone Grafts that support the bone and help it to rebuild itself again in a normal and healthy manner.

There are different kinds of bone grafts that can be used in any reconstructional surgical operations. The main function of this graft is to ensure the intuition of osteogenicity, osteoconductivity and osteoinductivity between the graft and injured bone[3]. Generally, orthopedists will discuss with this patient the different options of using any type of bone graft and tray to choose the most appropriate one. This option may be one of these types;

- **Autogenous bone grafts**, (or autografts), are composed of patient own bone tissue taken from typical site of his body like chain, hip or leg bone. The advantage of usage such graft that it contain natural bone structure with living cells, which improve the re-growth of bone in sufficient way.

- **Allogenic bone**, (or known as allograft), is a nonliving bone tissue taken from cadaver, used as a good framework in the bone tissue rebuilding processes. However, it does not have the ability to produce new tissue by its own, and in case performing of unsuitable sterilization it may be a source of infection diseases transmission to the patient.

- **Xenogenic bone** is taken from non-living bone of another species, like a cow. A high temperature is applied on this type of graft in order to get rid of any possible immunological and infectious drawbacks. Like the
allograft, it serve as good framework for bone re-growth in the site if injury.

- **Bone Graft Substitutes**
  Instead of using real bone, there are other types of graft that can be synthesized from safe materials and carry number of functional molecules to improve the bone rebuilding process, for example;

  - **Demineralized Bone Matrix (DBM)/deminerlized Freeze-Dried Bone -Allograft (DFDBA):**  
    This product derived from allograft; in a process that ensures removing the all the minerals from the bone and remaining the structural proteins with functional materials like growth hormones. It can be obtained in various form like jell, past and powder. It successfully used in the bone filling surgical operation particularly jaw socket extraction and in the complicated ununion bone fracture.

  - **Graft Composites:**  
    this type of bone graft contain different materials and growth factors to get benefit from different types of grafts substances. Some types of material combinations may be able to be found as: collagen/ceramic composite, which is very similar to the structure of natural bone. Also there is DBM combined with bone marrow cells, which aid in the growth of new bone, and the collagen/ceramic/autografts composite [4].

In the recent decade, we can easily notice the increasing number of studies carried out by biomaterial and bioengineering scientists in order to find more suitable bone grafts and scaffolds that not just mimic in its structure the natural building blocks of the bone tissue, but also the have the ability to carry various kinds of growth hormones and functional material that will improve the overall performance of the transplanted graft and give a high degree of satisfy to both patient and doctors. For this purpose, researches have done a lot of inspective studies on the human bone tissue to obtain very detailed picture that reflects the physiological and histological status that found in our bone tissue, and hoe it interact with any injury or damage that might happen in its structure.
Basically, bone tissue like other body tissue; consist of cellular compartment including Osteoblast, osteocytes and osteoclast. And noncellular compartment including the Bone Matrix. This specialized matrix consists of organic and nonorganic portions. The organic portion contain mainly collagen fiber type one that found in the shape of bundles with a diameter between 50 to 70 nm. Organic compartment also contain sulfated Glycosaminoglycans, like chondroitin sulfate and Keratan sulfate. These form small proteoglycan molecules with short protein cores to which the Glycosaminoglycans are bound. The proteoglycan are no covalently bound, via link proteins, to Hyaluronic acid, forming very large aggrecan composites. Glycoprotein like osteocalcin and osteopontine can be found in the bone matrix. They bind from a site to Hydroxyapatite and from another site with integrin presents on the surface of Osteoblast.

However, the nonorganic compartment which form about 65% of bone matrix, contain calcium and phosphorus along with other components, including bicarbonate, citrate, magnesium, sodium, and potassium. Calcium and phosphorus exist mainly in the form of Hydroxyapatite crystals.
[Ca10(PO4)6(OH)2], but calcium phosphate is also present in an amorphous form. Hydroxyapatite crystals are deposited into the gap regions of the collagen but also are present along the overlap region.[5]

After understanding the natural structures and building blokes of human bone tissue, scientist start to look for more feasible ways to form a highly functional synthetic scaffold structure that mimic for wide degree the natural form of bone tissue. Therefore, they used the newly emerged Electrospinning technology that gives the ability to form nanofiber structure which has the ability to serve as synthetic ECM in order to help the body during big tissue loss remodeling and reconstruction.

Electrospun nanofiber has different great outstanding properties that make its scaffold attracted big attention in the recent few years. Nanofiber based scaffold contain maicropours that help cell to migrate and mover easily and freely, with giving the chance to form suitable network of blood vessels inside it. the nanofibers have also any roll in cellular attachment and modulate cellular growth by releasing pre-loaded functional molecule such as growth hormones and factors in order to mimic the general roll of bone matrix, beside to the biologically functional molecules that can be loaded, nanofiber also has the ability to act as Drug Delivery System in order to carry drug molecules and ensure protection and also continues releasing of these drug to the site of wound, like antibacterial drugs and anti cancer drugs.

![Image](image_url)

Figure 1.3 Compairson between (a) normal tissue histological structure and (b) nanofibers based scaffold structure

In the recent few years, an increasing number of anticancer drugs carried by nanofiber scaffolds start to be noticed. These drugs have different chemical
structure derived from different sources. Some of them synthetic like cyclophosphamide and methotrexate. And the other can be categorized as natural or herbal drugs like Baicalein and Baicalin. These Chinese herbal drugs are commonly used as in the traditional medical therapy and nowadays are believed to have an antinflammatory and ant cancer effects against number of cancer diseases. These crucial properties make the scientist to do further research in order to explore their actual effects and try to get advantage form them to treat different types of diseases.

In this study to apply the Electrospinning method in to produce nanofibers driven from both natural polymer which hyaluronan acid(HA) and synthetic polymers ; Polyethylene oxide (PEO) and polyvinyl alcohol (PVA) loaded with TGF-beta and Baicalein drug material in order to form functional nanofiber based scaffold which posses anti bacterial and also anticancer properties that can destroy any tumor cell remnant after any wide –excisional or even therapeutic palliative cancer surgery that result in bone tissue loss .
2. GENERAL INFORMATION

2.1. ELECTROSPINNING AND NANOFIBER SCAFFOLDS

Nanofiber is a nanometer-scale structure, composed mainly from polymer materials with theoretically unlimited length and a very small dimension at about 100 nm or less. [6] These tiny parameters give the nanofiber unique characteristics such as large surface area in comparison with volume, and having a high level of molecular binding affinity with its surface functionalities [7]. These special properties aid the nanofiber to have different application areas in a large number of fields that affect our everyday live.

A number of synthesis methods such as drawing [8], phase separation [106], template synthesis ([45] and [108]), self-assembly [9, 10], Electrospinning ([29] and [49]), etc. have been used to prepare polymer nanofibers. Drawing method, for instance, can make individual long single nanofibers; by which the material used in nanofiber formation should have viscoelastic properties that help them to undergo strong deformations while being cohesive enough to support the stresses developed during pulling forces throughout this process. On the other hand, phase separation method includes dissolution, gelation, and extraction using a different solvent, freezing, and drying techniques resulting in nanoscale porous foam. The template synthesis method includes the usage of a nonporous membrane as a template in order to make nanofiber of solid (a fibril) or hollow (a tubule) shape. The importance of this method may lie on the fact that; nanometer tubules and fibrils of various raw materials such as electronically conducting polymers, metals, semiconductors, and carbons can be synthesized. However, the method cannot make one-by-one continuous nanofibers that can be seen as one its disadvantages. The self-assembly, on the other side, is a process in which individual, pre-existing components that can organize themselves into desired shapes and functions. But in the same time we can say that this method can be considered as a time-consuming processing in terms of continuous polymer nanofiber formation. Consequently, the Electrospinning process seems to be most suitable method that can be used for mass production of one-by-one continuous
nanofiber from a wide range of polymers.

2.2. Electrospinning

In 1993 Renker had defined the Electrospinning concept as a kind of fiber synthesis processes in which different types of forces are used in a way that initiate and control fiber production [11]. Over through the last two decades, Electrospinning method had attracted the attention of scientists from different aspects and specialties, since it has the ability to produce nanostructures and nanofibers that have either solid or hollow lumens with numerous properties such as continuous length, adjustable diameter, versatile, and controllable components [12] and [13]. In a way that differs from other methods, the technique of Electrospinning depends on the effects of electric field that applied on a drop of previously prepared polymer solution in a way that makes a jet of charged polymer mixture to elongate and form nanofibers with desired characteristics and properties [14] and [15]. Electrospinning can be used to fabricate nanofiber structures form various types of polymers such as natural and synthetic ones, polymer alloys, and eventually polymers with functional nanoparticles, that gives Electrospun fibers such wide range of applications to be used through all our lives aspects.

2.2.1. History of Electrospinning

By looking through the text books and old papers searching for the beginning of Electrospinning techniques, how it appeared and what are the milestones through which this method has appeared and developed, we can notice the great amount of strenuous efforts that have being made toward the achievement of fiber formation with both tiny diameter and high degree of strength. These trials have been started in 1628 by the reporting a preliminary explanation of electrospraying phenomena by the English physician, physicist William Gilbert, who described his observation about the effect of electrical field on a water droplet that makes it take a conical shape and eventually a small droplets will be ejected from the tip of the water cone [16]. In the same way of simple and preliminary experiments, C. V. Boys et al. had made a very simple spinning apparatus consisted of an insulated dish connected with an electrical machine. Boys found that fibers-like structure have been formed on the dish by using stocked materials
like beeswax and collodion. The scientists like Gilberts, Boys and others continued doing a lot of trials in this field till the beginning of twenty one century when the first patent was granted to J. F. Cooly in May 1900 and February 190 [17, 18].

In 1914 J. Zeleny by reporting the behavior of fluid droplets at the end of metal capillaries in the presence of electrical field.[19] His attempt could be said the first the effort to understand mathematically the behavior of liquid solution under electrostatic forces. However, the Electrospinning, as a standard technique for textile nanofiber formation, has been invented by Antonin Formhals in 1934, who granted a patent on making the first Electrospinning apparatus that could produce fibers size from less than 100nm to tens of micrometers. This invention had played quite a big role in terms of commercialize the textile yarn formed from Formhals's nanofibers.[20]

In the period between 1964 and 1969 G. I. Taylor has successfully put the theoretical mathematical explanation of Electrospinning.[21, 22] Taylor’s work engaged in modeling the shape of the cone mathematically, that formed by the liquid droplet under the effect of an electric field; this unique droplet shape is called now “Taylor cone”. Taylor further worked with J. R. Melcher to improve the "leaky dielectric model" used in the process of conducting fluids.[23].

In the last decade numerous research groups (Renker et al. for instance) and academic institutions reported that we have the capability to synthesize nanofibers from various organic polymers. this outbreak, if we can call it, made the number of papers in this field to increase dramatically every year.

2.2.2. Apparatus And Method

In order to get a fine and suitable nanofiber via Electrospinning process, a high voltage source is used to form a polymer solution or an electric charge jet in the melt of a prepared polymer solution. The polymer solution is being prepared by dissolving the polymers row material (in a powder or gelatin state) in good solvents until being dissolved homogenously and completely within the solvent. After that a glass tube or a syringe is used to be the container of the polymer solution that will be sited on an electronic syringe pumper which allows the solution to flow at a constant rate across the syringe needle. One of the electrodes is connected to the
syringe needle, while the other electrode is connected to a rolled collector that is suited in front of the syringe.

It’s believed that the principle of Electrospinning is mainly depending on applying an electric field to the tip of the syringe loaded with the polymeric solution. The main goal of applying such an electric field is to overcome the surface tension of a polymer solution droplet let that formed in the tip of syringe by the controlled pumping process via the electrical pumper. Consequently the body of the solution droplet becomes charged, and the electrostatic repulsion force starts to counteracts the opposite surface tension till the droplet begin to elongate and take a stretched shape, and at a critical point, a stream of liquid erupts from the surface taking a unique conical appearance that is known as the Taylor cone.[24] Eventually, when the molecular cohesion of the charged liquid is quite high, a stream of charged jet will be shot out of a needle toward a conducting collector. The volatile solvent evaporates in the air leaving behind, under the right conditions, a polymer fiber with a diameter that can range from tens of nanometers to microns [12]. Collected as nanofibers as shown in Figure 10 [17].

![Figure 2.1 Electrospinning Setup](image)

The usual intents of scientific models apply here, such as to help us to understand the process, control the process, or to improve upon process limitations. All of that gives the needs to have both a good knowledge and also a theoretical prediction.
to understand the real effects of the nanofibers fabrications setups. Or what are known as Electrospinning Parameters.

2.2.3. Importance of Process’s Parameters

Nanofiber formation process, like any other fabrication domains, has a distinct standards and parameters that might have changing and variations depending on what type of polymers used during such process, and how the electrical field will act on the droplet of the polymers solution to form the unique structure of Taylor cone, then causing a jet of charged solution to shut out and settled down on the collector surface in a form of nanofiber. [25]. This complicated and overlapping procedure needs to be controlled precisely and accurately, otherwise we will face different sorts of difficulties and troubles like getting a non reliable spun nanofiber diameter and/or diameter distribution. As well as having some obstacles in controlling nanofiber morphology, diameter uniformity, formation or absence of beads related to capillary instability.[26]

Recently, many empirical observations indicated that any formation trail of synthesis nanofibers using Electrospinning apparatus require a fine control to number of parameter such as solvent properties, solution flow rate, voltage, distance from needle to collector, polymer concentration and other important parameters [11, 27-34]. For instance, Fong et al. reported that during PEO nanofiber formation, both the fiber and beads diameter tend to increase with the increasing of polymer solutions flow rate[35]. Whereas, Jarusuwannapoom et al. noticed that an increasing in needle – collector distance will dramatically cause the Polyesteren nanofibers diameter to decrease, and in the same time any formed beads, if there are, will have tendency to grow and enlarge.[36]. Furthermore, Zuo W et al. observed a kind of decreasing in the poly(lactide-co-glycolide) (PLAGA) nanofiber diameter in correlation with increasing in the electric potential from 8 to 10 kV.[37]. In terms of polymer concentration and its effect on the nanofiber formation, we can noticed number of papers that mentioned the corresponding relationship between the amount of polymer used in the process and the end result nanofiber shape and diameter. However, although the clear bond between the percentage of polymer used and its nanofiber, the variation attitude that can be observed in the values of these tow variable does not take a constant shape. In another ward, tiny alteration to the polymer concentration may
result in quite obvious impact on the formed nanofiber, while sometimes the polymer percentage needs to be change in wide manner in order to get even a small improvement in the nanofiber configuration, that’s will be more if we have a look on what Chass GG et al. has described the relation between Naylon 6 polymer concentration and the final cress sectional radius and its resulted nanofiber diameter as a steady linear relation Fig.[32] on the other hand, we find Wendorff, J.H in his book[38], for instance, taking about a nonlinear fashion between the former tow variables during the polyacrylonitrile nanofiber formation as shown in fig. that’s reveals that at a distinct polymer concentration value any small change will result in a quite clear alteration in the resulted fiber structure. Fig [38]. This clear diversity in the resulted nanofibers is can be attributed to the difference in the viscous and viscoelastic properties of polymer solution that dramatically will vary with any change that might happen to the concentration of the polymer materials in the solvent.

As a result, we can find that Electrospinning process has number of parameter that have a different effect on the fiber formation, and this parameter is summarized as Gupta et al. mentioned in his paper [25]:

- Solution parameters; Conductivity, polymer concentration, solutinal surface tension, viscosity.
- Process parameters; the applied electrical voltage, the distance between the tip of the needle and the collector surface, polymer solution flow rate, the electric field.
- Environmental factors; humidity and temperature.[25]
Figure 2.2 Calculated final jet cross sectional radius by the changing in the Polymer concentration

Figure 2.3 : Fiber Spun From Different concentration(a) 8%wt solution resulted in fiber of 300 nm and (b) 12%wt solution resulted in fiber with 750 nm in diameter (From D. Hussain, PhD Thesis, Department of Chemistry, Philips Universitat) (Marburg 2009)
2.2.4. Nanofibers’ Component Materials

Generally, most of the fibers that are being synthesized via Electrospinning method are made mainly from polymer materials. For special purposes, sometimes we find that scientists intend to add other materials like some kinds of drug molecules like antibiotics [39] or anticancer drug [40], and also different sorts of nanoparticles like silver nanoparticles [41] and Platinum nanoparticles [42]. Although the impact of these small particles and supportive materials is quite crucial especially to the aim for which the nanofiber have being formed (anticancer or antibiotic effect for instance), but still the polymer molecules have the big impact on nanofiber formation, and because of this we will take about two types of polymers that being used in the process of nanofiber formation via Electrospinning, and have been applied in a wide range of life field, they are Hyaluronic acid (HA) and Polyethylene oxide (PEO).

2.2.4.1. Hyaluronic Acid

Hyaluronic acid (HA), or what is occasionally called hyaluronan, is one of the negatively charged, no sulfated Glycosaminoglycans (GAG) molecules that can be found in different sites and organs of our bodies mainly the connective tissues and joints. Its unique chemical structure and consistency gives it a remarkable
peculiar properties that makes the molecule of Hyaluronic acid a crucial supportive component to the surroundings cells and ECM.[5, 43]

The discovering of Hyaluronic acid was done by the year 1937 by the German biochemist, Karl Meyer, and his assistant John Palmer. At that time they reported a method of isolating a new Glycosaminoglycans (GAG) molecule from the viscous vitreous humor of the eye. They showed that this molecule consisted of an uronic acid and an amino sugar but without sulfonic esters, and as a result of that they chose to use the term of ‘Hyaluronic acid’, from hyaloid (vitreous) + uronic acid.”[44] This was the beginning of Hyaluronic acid researches that revealed as well will mention later, the big roll that such a GAG molecule can have in different sites of not just human body which consists a 7 g to 8 g to the adult human average weight, but also it plays the same important roles in various types of animal species.

Hyaluronan or Hyaluronic acid has a unique chemical structure that gives it the ability to achieve a number of vital functions. Theoretically, HA is a linear polymer consists of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid which connected through glycosidic bond. These units linked with each other in a big number to form the HA with a molecular mass in range between 104 and 107 Da. It is distinct from other GAGs (Heparin- heparan sulfate - chondroitin sulfate - dermatan sulfate Keratan sulfate) that it neither possesses any sulfate group in its own internal structure, nor it connects covalently to any peptide molecule.

Figure 2.5. Hyaluronic acid
Emerging roles of Hyaluronic acid bioscaffolds in tissue engineering and regenerative medicine

From this unique structure we can predict that HA may have numerous chemical and physical characteristics that make it plays a very important role within different types of organisms. The high viscosity of HA solutions, that Necas et al. described it as it’s high than what’s expected, and in the same time being able to be sheared and injected through a thin needle even in a small concentration percentage (1% for instance). As a result, HA can behave as a “pseudo – plastic”, and consequently, it can be applied as a lubricants materials in different biomedical applications.[45, 46].

Also the relatively high hydrophilic properties that HA has gives it the ability to make a relatively big number of bonds with water molecules, that helps the HA to obtain the rheological properties that can be noticed in several biological applications.

However, in parallel to the chemical and physical characteristics that HA has, it also possess an essential importance in terms of cellular and tissue supporting through the unique performance that can clearly be noticed at different site of any organisms body. Since the chemical structure of HA that permit it to form bonds with water molecules as well as some kinds of cations such as sodium atom, a great amount of extra cellular fluids can be attracted by HA - (Na+) complex forming a strong hydrates structure that plays a big role in terms of compression forces resistance. Moreover, by arranging these molecular complexes close to each other, the negative charge they posses makes them to repel one another, giving the overall structure a slippery-like property that can be observes in numerous sites in our bodies like synovial fluid in the joints and vitreous hummer gel in the eyes [5]. The application of this properties can be observes clearly by taking a look at the relationship between HA and Aggreca, one of the most Proteoglycan macromolecules found mainly in cartilages and connective tissue proper. Such an attachment between these two molecules could reveal an essential roll terms of forming a stable complex that give the ECM to have a gel state acting as a natural barrier against any diffusion of unwanted aqueous martial and deposits. Shape() [5].
Furthermore, HA has a regulatory function, particularly at the cellular level by interacting with specific receptors found on the cell surface. These receptors like CD44 (cell surface glycoprotein, implicated in the stimulation of cellular aggregation, proliferation, migration) [47, 48], and RHAMM (receptor for HA-mediated motility, playing a role in cellular responses organization to multiple growth factors, as well as cell migration) [49, 50], seem to have received more attention, since they considered as the bridging molecules that make the HA to effect on the cellular cytoskeletal protein and therefore has the ability to get access even to the cell nucleus by specific signaling processes that can create an impact on genomic translation within specific kind of cells. [51]. The HA has a significant role in the immune response against bacterial invasion through its activation of specific type of WBC such as macrophages and dendritic cells by binding to one of the Toll-like family receptors (TLR), and consequently initiation innate immune response [52, 53]. In the same time HA can be an essential factor for both initiation and suppression of any inflammatory event. For instance, binding of HA to CD44 or TSG-6 receptors can provoke an inflammatory onset by increasing the number of specific pro-inflammatory cytokines such as TNF-α and IL-8 [54]. However, HA can be also considered as a negative feedback factor through any inflammatory process. this mission can be achieved by the mean of its own radical scavenging and antioxidant properties. [46]. In addition to that, HA has a great capability to influence the tissue remodeling and repairing after any damage by activation cell proliferation like what’s happening to fibroblast mitotic satiate that increase in respond to the impact of HA [55, 56]. this process can be clearly observed in terms of epithelial wound healing, in which the HA will make first the keratimicytet to proliferate in an accelerated rate, then it would promoted to migrate to the newly formed ECM as result of CD44 effects on cell migration and movements. [57, 58]. By the ending of re-epithelialization step. Wound healing process will be ready to what it called wound remodeling at which in some cases a big amount of scarring could take place, but in terms of this some studies have reported that the presence of HA or its fluid in the wound area may minimizing the scarring formation ant the stretched that can may noticed in such wounds to a great degree, thereby it can improve tissue healing processes as well as decreasing scarring and fibrosing deposition. [59, 60]
2.2.4.1.1. Application of HA

Hyaluronic acid can be modulated to form covalent bonds with various antimicrobials molecules and therefore make an antimicrobial coating solution or gel that shows a significant effect against some kinds of strong antibiotic resistant bacteria such as Staphylococcus *epidermidis*, Staphylococcus *aureus* and Pseudomonas *aeruginosa*. [61]. As an application to this conception Giammona et al. has reward a patent in the field of hydrogel – Hyaluronic acid dependent material that can be used in Prosthetic surfaces coating. This invention has revealed the high capacity of such hydrogel to be mixed with numerous kinds of antibiotic, vancomycin for instance, and use it as a covering surface at the sits of prosthetic par that may have relatively high probability to develop bacterial growth on them. And as expected to play an important role in prevention and/or curing orthopedic postsurgical infections by forming a kind of coating film helps in lubricating the articulating area as well as improving the defense properties against differ types of antimicrobial resistant bacteria. [62]
Diabetic foot ulcer (DFU) consider as one of the most popular complication of Diabetes mellitus that immerge by the presence of both neuropathy and vasculoplasity that can be found in the most cases of chronic phase of DM patients [63]. in case of untreated ulcers, some of DM patient may suffer from divers levels of complication that may leads to do amputation to the entire leg . [64] as a result of this problem , scientist have developed a spongy- like wound dressing used chiefly for DFU treatment that composed of chitosan, Hyaluronic acid (HA), and nanosilver (nAg) . this invented dressing showed a good results in terms of ulcer curing , as well as prevention of bacterial growth especially the types that considered as antibacterial resistance ex; methicillin-resistant S. aureus, Pseudomonas aeruginosa, and Klebsiella pneumonia . [65]

In Osteoarthritis disease that can develop in up to 70% of the 70 years old people, and characterized by degeneration and debilitating of the cartilage surface located in the diarthrodial joints. a study was made by Forsy et al. showed that HA application inside the affects joints with osteoarthritis disease can improve the remodeling of the fractured or damaged cartilage surface by direct interaction with condreocyte that found in the area of fracture . such an application of HA can be very helpful for a big number of patient suffering from osteoarthritis complications[66].

In addition to that HA also has been reported to have a strengthen effect on the immunosystem organs such as spleen and thymus by its roll in splenocyte proliferation, and increasing the efficacy of the peritoneal. Furthermore the HA also showed an antiangiogenic activity when it applied in vivo animal model according to a study carried out by ke et al. when he used a low molecular weight Hyaluronic acid . and as a result of this he suggest that that Low Molecular Weight HA might be one of the potential immunomodulator and an essential candidate compound for antiangiogenicity.[67]. Moreover, HA have been used in association with cardiovascular implants as it considered an antiadgesive compound particularly with its ability to decrease platelet adhesion thrombosis at vascular grafts sites and heart stents. [68]. Number of Recent studies are talking about the role of HA not just a supporting compound for ECM or even a natural material to cure number of diseases , but also they mention that that HA can bind to magnetic silica microspheres to form a novel compound that has a potential
application in terms of cooper removal from the water, such a thing can have a big outstanding effect in maintaining and preserving our Environment.[69].

the putinyaila function of HA can be noticed clearly in the field of cancer treatment. Many studies have obtained good results against different type’s cancer cell through a successful synthesizing of anticancer loaded nanoparticles that has a relatively high capacity to such dugs. Although these nanoparticles have the liability to sustained releasing of loaded dugs, however it didn’t reach the targeted cancer cell properly due to some physiological obstacles. This issue brightened out the need to use such a molecule that can increase the targeting liability of anticancer loaded nanoparticles by binding specifically to the target cancer cells without any effect on the surrounding normal tissue cells. for this purpose Saravanakumar et al. has used one of Amphiphilic HA derivative that has the both the ability to maintain a sustain release to the hydrophobic PTX anticancer drug molecule, as well as the high affinity to bind with CD44 receptors that are known to be over expressed on the cancer cells surfaces. And therefore he could achieve a successive reliable targeted cancer cell therapy.[70]. In parallel to cancer treatment, the diagnostics tools and follow up method regarding any treatment process to cancer disease had got benefit from the relationship between Ha production and cancer cell sit self. Scientist have noticed that there’re a massive production of Ha in tissue surrounding the cancerous cells as well as the cancer cells itself due to an over expression of the hualuronisc acid in such cancer cells [43, 71].According to this revelation, number of reports have talked about making some trials regarding the prediction of cancer cell in the body depending on estimating the amount of HA produced in the surrounding tissue[43]. Similarly, Lokeshwar et al., reported that the measurement HA concentration exposed with urine can be of great benefit regarding the detecting and estimating the status of bladder cancer.[72]

2.2.4.1.2. HA based scaffold application

In recent years there has been increased attention to the usage of HA particularly in the field of scaffold formation and tissue engineering. Since the potential properties that HA hold such being one of the biocompatible and biodegradable materials that FDA has approve it to be used in the food and supplement materials. For this purpose a respective number of studies have reported that HA
usages and application has became one of essential compound the used in scaffolds formation. One of the most prevalent application that can be mentioned regarding HA is being a Space Filling Implants And Scaffold Compound that possess respected number of applicable fields such as urinary incontinence treatment [73], somatic procedures[74] , and plastic and reconstructive surgery [75]. furthermore, the Hyaluronic acid had been used successfully in the branch of nanofiber based scaffolds formation depending on its vital properties that make it one of the essential materials that can be get used in the area of implant fabrication via Electrospinning technique. most of the time, HA was not used as a pure consistence for the production of nanofiber, indeed the, HA was mixed with other material I order to get a high quality fibers with multiple functional capability, and In the same time, the spinning mixture was loaded with drug molecules or hormonal compounds which makes the resulted scaffold one of the important Drug Delivery Systems. Here are some recent example of HA based scaffold application; Ahire et al. (2017) reported the spinning of blended HA with polyethylene oxide (PEO) In order to form antibacterial scaffold served as joints implant coater with antibacterial function [76]. Enntehkabi et al. (2016) designed a highly porous scaffold for neural regeneration using a mixture of HA and polycaprolacton (PCL)spinned nanofibers [77]. Also the blended mixture of PCL/HA was spun by Chen et al. (2015) and loaded with silver nanoparticles. the resulted scaffold was applied successfully in the tendon surgery operation aiming to prevent tendenous adhesion, providing suitable lubrication and minimize bacterial invasion to the wound site [78].

2.2.4.2. Polyethylene Oxide

Polyethylene Oxide or (PEO) is one of the synthetic polymers that has gained a tremendous attention in various fields and particularly in terms of tissue engineering (TE) and Drug delivery systems since it posses number of properties that make the scantest prefer to use it in their biological and TE projects and Experiments[79].
Indeed, PEO is considered as non-ionic linear molecule that formed by ethylene oxide polymerization process. The atomic structure can be expressed as H−(O−CH2−CH2)n−OH, which gives it the semi crystalline structure shape [80].

PEO has a unique set of features that make it a highly used polymer in different processes. One of the main features that PEO possesses is being both biodegradable and also have a good solubility in both organic and aqueous solvents. Moreover, PEO believed to be safe, biocompatible compound and very suitable material to be used in the field of Tissue Engineering and biological implants [80]. Further potential feature such as; viscosity, surface tension and conductivity give PEO a great ability to be used in various fields and for different purposes. Cerchier et al. reported the great advantage of metal coating with PEO which can be represented by improvement in the both wear resistance and also the corrosion properties of metal, or award additional functional features like anti friction and thermal protection [81]. Furthermore, PEO can be used in the field of pharmaceutical industry in terms of drug delivery carriers and vehicles, that has the ability to swell and become a hydrogel in the presence of gastric secretions, so that it can serve as a controlled vehicle for drug releasing process[80].

2.2.5. Application of Nanofiber Formation

2.2.5.1. Lithium-Air Battery

One of the electrochemical devices that used mainly for energy storage is called Lithium-air battery that has gained a great attention because of the tremendous capability for storing energy and possessing high power density[82]. In normal conditions, Lithium ions are directed to combine with the free Oxygen molecules to form LiO2, transversally when the battery being recharging, Lithium ions will
dissattaches from the oxygen molecules leaving them to be released back to air. These two processes of charging and discharging have number of adverse effects on the batteries structure, performance and also the overall life span. thus, in order to overcome these obstacles, Zhu et al. has developed a new kind of betters that contain Carbon nanofiber body as a Cathode, which has the ability to posses the two functional elements; Lithium and oxygen by the presence of Cobalt Oxide that give the needed stability support. since the highly porous structure of carbon nanofiber that give high degree of carrying Lithium and oxygen molecules that will get in to the charging and discharging processes by transit between the Li$_2$O$_2$, and Li$_2$O. Thus keeping the oxygen in one solid stets rather than being converted in to gas state in every time the battery being handeld in charge. [83].

2.2.5.2. Filtration

Many nanofiber products have been developed in order to be used in the field of Air & Atmosphere filtration. One of the examples that shows the application of the novel nanofiber structures is the development of Polyurethane nanofibers by Scholter et al. [84]that can play a big role in terms of volatile organic compounds (VOC) clearance from air like Hexan and chloroform compound, that have a large harmful effect on the humankind health status. By getting advantage from the adsorbent ability of Polyurethane particles to various type of organic liquids and vapors [85, 86], and the high surface area that polyurethane nanofiber can posses, schotler et al. proved that polyurethane nanofiber membrane could remove a high percentage of multiple VOC from the surrounding air, and thus increasing the percentage of clean at atmosphere in both indoor and outdoor areas.

Moreover, Ma Wenjing et al. reported in his study the production of the core/shell Polyamid acid/Cellulose acetate nanofiber membrane with the presence of both fluorinated polybenzoxazine and silica nanoparticles. This new products of filtration membranes has a big capability to be implicated in the development of oil-water novel separation technology, thus it can be utilized to meet the need of big numbers of governments and petrol companies that may exposed to an oil splitting accident and thus have the responsibility to take a step against any environmental critical case that could happen. Wenjing reported in his project the properties of his filter product that came as a results of bringing different kinds of elements and blended them together in a form of nanofiber membrane that posses high
hydrophobic (fluorinated polybenzoxazine), good resistance to heat and corrosion (Polyamide acid), high degree of superoleophilicity and elasticity (Cellulose acetate) and labeling to control the wettability by modifying the surface structure (presence of silica nanoparticles) [87]. The results of his published study was a successful production of nanofiber membrane with an high oil-water separation ability (<99%) and also a high flux velocity (3106.2 ± 100 L m⁻² h⁻¹).

![Image](image_url)

**Figure 2.8. SNP/FCA Filteration Membrane**: The membrane demonstrate a n oil-water separation properties (after 30 seconded the e water remain upside, while the oil crossed the membrane to fall down the bahar.[88]

An in situ polymerization approach for the synthesis of super hydrophobic and superoleophilic nanofibrous membranes for oil–water separation

### 2.2.5.3. Coating and Packaging

Since the big advantages that electrospun nanofibers show in multiple fields and areas, a considerable number of studies have been carried out in order to look for any possibility to apply this new technology in the textile coating and food packaging sectors. So in the terms of this topics, we find Jianfing et al. reporting in his study a successful production of a waterproof – breathable nanofiber fabric that has both hydrophobic and oleophobic properties with WCA of 156° and OCA of 145°, yet it allow to the water vapor molecules to pass throw its interconnected porous structure. He mentioned that utilizing of PU (Polyurethane) connected with perfluoroalkane segment may result in a fluorinated PU complex (FPU) that has a low surface energy in contrast to PU alone which posses a high surface energy.
This precious property of FPU makes the produced nanofibers not just have an outstanding waterproof potency, but also it will possess a micro porous infrastructure that permit the H2O vapor molecule to pass through it to other side. [89]

![Figure 2.9. Using Polyurethane / Polyethylene Nanofiber Film for Breathable Waterproof fabric formatim [90].](image)

This new method can be used in various field for different kind of purposes, for example: NBC (nuclear, biological, chemical) protection suit fabrication for Military application, Bacterial and viral barrier for wound dressing and healing, nanofabrica application as antistatic and soil resistance layer, bioseparation and nano particle filtration.

Another application of spun nanofibers can be found in a study carried out by Fabra et al. as he reported the production of biodegradable food packaging material by utilizing of thermoplastic corn starch (TCS) with bacterial cellulose nanowhiskers (BCNW) to form a kind of nanobiocomposite that in further steps will be coated in multiple layers of poly(3-hydroxybutyrate) (PHB)/BCNW electrospun nanofibers which will provide the overall with number of potential characteristics like being a transparent multilayer structure with both high impermeability to Oxygen and water, and also have a considerable degree of elasticity with good interlayer connection conditions. [91]
2.2.5.4. Cancer Diagnosis

In order to give successful treatment procedure, most of the cancer diseases have to be detected in such a way that provide a high degree of sensitivity. Although the pathological examination is considered as a golden standard method in terms of cancer staging and diagnosis [92], still the scientist looking for more quickly and simple invasive procedure that can be done for cancer patients in both cheap and comfortable way. One of the good example for simple cancer detection methods is a study that carried out by Wang et al. (2015) in which he reported the production of multi walled carboxylated carbon anhydrate (MWCNT) - nylon 6 (PA6) composite Electrospun nanofibers based electrolytic immunosensor to detect Tumor Suppressor Protein P53 that in most cancer cases, may have some genetical mutation, thus any measurement for P53 may lead to an early prediction for a big number of both benign and also malignant cancer. By getting advantage of the considerable large surface area of CNT and the excellent semiconducting of PA6, also by the addition of (Thioinin) as a principle element for electro polymerization process, the formed structure has been ready to serve as detector for P53 Antibody (AGp53) and for further measurements [93]. In the same context, Paul et al. (2016) showed in his study the ability to d to form the basestructre for biosensonelctrod on which he immobilize the anti-CA125 antibodies that will bind
to the CA-125 antigen and produce an electrical signal that will be conducted through the MWCNT – ZnO complex nanofiber and being translated as a measurement in order to detect the presence of any ovarian cancer cell and making further treatment and follow up for this case.[94]

Figure 2.11. Schematic representation of the fabrication of carcinoma antigen-125 immunosensor [94]

However, there are other type of studies that show the possibility to detect the presence of cancer cells particularly those that had been shed to the blood stream in case of malignant cancer sources. Circulating tumor cells (CTCs) detection, nowadays, can be made through a new procedure called Liquid biopsy which considerable as and the prognosis of the malignancy [95]. Not long ago, Ke et al. (2015) reported the production of very promising chip called Nano Velcro that can capture the CTC from the blood stream and analyze it. It has passed through 3 generations; the first generation was for cancer cell detection and staging, the second was developed for single cell isolation and genotyping, while the third generation was design for more advanced CTC purification. by using the poly(lactic-co-glycolic acid) (PLGA) electrospun nanofiber coated with anti cancer protein found on the surface of the cancer cells, this structure will be applied to a
UV laser beam for isolation and detecting processes

Figure 2.12. NanoVelcro Chip for Circulating Tumor Cell (CTC) detection

2.2.5.5. Tissue Engineering

In 1985, the term “Tissue engineering” was first used by Y.C. Fung to describe the interdisciplinary field of science that involves material science, life science, medical science, and engineering aiming to provide a very suitable solution to the most health issue that may happen to the organic tissue of the human body and help it to overcome a wide range of obstacles that prevent it to regulate itself and being well again in normal position[96]. However, tissue engineering, like other applied science fields, has several obstacles that may impede if not preclude the process of interaction between the used material from one side, and the biological tissues and organs from another side. These obstacles and difficulties like lack of capillary system inside the scaffolds or even the high price and expensive materials being utilized [97, 98], all of this drawbacks has begun to be replaced with number of potential solutions that possess a high degree of outcome and advantages which can be applied efficiently and effectively in various sectors of TE. Nanotechnology and especially Electrospun nanofiber has attracted the attention of most TE scientist because of the greater opportunity that offer in order to find more practical solution for the difficulties that we mention former in this context. Usage of nanofiber scaffolds has been used for a wide variety of TE
applications like Tissue grafting, Wound dressing and Drug carrying and releasing.

2.2.5.5.1. Tissue Grafting

Nanofiber based scaffolds structures used widely in the tissue engineering because the unique structure that mimic the natural temple and composition of the body Extra cellular matrix [99]. Moreover, during fabrication process, these nanofiber dependent scaffolds be provided with various kinds of particles that play a big role in terms of increases the regenerative ability of the damaged tissue [100]. For instance, in the bone regeneration context; adding willemite nanoparticles, which are special type of crystal composed of both zinc and silica elements, to poly (l-lactide acid) (PLLA) nanofibers has showed a high degree of osteoblastic enhancement, Osteoblast protein and gene expression and also bigger ability to initiate osteoblastic differentiation to the injected stem cells inside scaffold structure [100]. Furthermore, Wang et al. (2017) demonstrate in his study a novel way for tracheal cartilage loss or damaging caused by trauma, cancer or even stenosis which has no real treatable manner. So he mentioned the use of Mesenchymal stem cells (MSC) derived from Wharton’s jelly of Umbilical cord that have a high ability for cartilage specific gene expression [101] with core /shell bovine serum albumin (BSA)-rhTGF-β3 / P(LLA)-collagenelectrospun d that happens in a high rate above the 65 years old [103]. The study demonstrates the formation of multi layers Polycaprolacton (PLC) nanofiber along with polydioxanone (PDO) nanofiber that has been attached to woven fabric of PDO which has a high compatibility with Tendon cells [104], along with good suture pull out strength during surgery. These novel nanofiber scaffold has tested for in vitro and in vivo test,, and the results showed a good degree of cellular infiltration through the scaffold layers with suitable strength that mimic the natural rotator cuff tendon and with no inflammatory area rejection sign during in vivo transplants.
Moreover, the cardiac tissue regenerative treatment that aim to reduce the tremendous drawbacks of the coronary heart diseases complication and particularly Myocardial infarction (MI) in which the cardiac tissue has a severe damage due to d field of Electrospun nanofiber scaffolds. One of the most recent studies reported the usage of Mesenchymal Stem cells embedded in PCL nanofibers structure loaded with Vitamin B12 which help in reducing the heamocystin level and promoting cardiac beating cell differentiation [106]. PCL nanofiber was also loaded with gold nanoparticles which had showed potential antioxidant and anti inflammatory effects[107]. MSC driven from bone marrow has the ability to differentiate into mature cardiac cell in the presence of natural heart ECM which has been offered by embedding small number of Cardiac cell in the scaffold structure. The results of these study showed a remarkable improvement in the MSC’s which had the ability to produce some kinds of unique cardiac cell proteins such as Troponin – T and Actinin. Thus it can be considered as a
promising heart graft for the treatment of damaged cardiac tissue in terms of MI cases.[108]. The TE scientist also have used the nanofiber scaffold for another critical cardiac case which is heart valves damage in which they try to find regenerative solution to repairing or even replacing the nonfunctionized valve with synthesized biocompatible one. in this context Fallahiarezoudar et al. (2017) has carried out a study about formation of synthesized a complete heart valve with leaflets and roots from poly-L-lactic acid (PLLA)/thermoplastic polyurethane (TPU) loaded with Maghemite ($\gamma$-Fe$_2$O$_3$) nanoparticles which has been reported its biological effect on cell regeneration and proliferation. Along its good mechanical effect on the scaffold strength and duration. It’s worth to be mentioned that the high porosity of the formed valve structure along with its elastic properties that possess, could be a promising valve regenerative graft, particularly when we recognize that Human aortic smooth muscle cells (HAOSMCs) used in the in vitro test had the capability to infiltrate in to the valve structure and proliferate in good way.

![Figure 2.14. 3D Scaffold Of Aortic Heart Valve Including Leaflets And Root](image)

Figure 2.14. 3D Scaffold Of Aortic Heart Valve Including Leaflets And Root
However, regenerative neural tissue engineering has made great strides especially in neuronal damage or complete neural tissue loss, there are considerable number of studies had carried out by getting advantage from electrospinning technology aiming to mimic the natural neuronal ECM that will play a big role in re-proliferation of neuronal cells. For instance; Zhang et al. (2015) reported the production of blended electrospun micro/nanofiber composed of Lysine-doped polypyrrole (PPy) as a core (which has good biocompatibility with high conductivity particularly for Electrical stimulating during neural cells regeneration [109]), with both PLLA and regenerated spider silk protein (RSSP) (which has low biodegradability with good bioconductivity suitable for neural regeneration [110]) used as a shell layer. The results of this study revealed that PPy/PLLA-RSSP fiber scaffold had good biocompatibility and cell adhesion with PC12 cell with production of cell-neuritis or neurit-neurit connection within the scaffold structure. Moreover, in vivo test showed the ability to bridge 2 cm loss in the sciatic nerve of rabbit, along with application of electrical stimulation to the scaffold structure that leaded to advancement schwa cell growth [111].
Figure 2.16. Representative Confocal Laser Scanning Microscope Images Of PC 12 Cells Cultured For 3 Days on (a) L-PRPN Composite Micro/Nano-Fibrous Scaffold, (B) On Control [111]

Figure 2.17. Re-growth Of Neural Tissue After L-PRPN Composite Transplantation

2.2.5.5.2. Drug Delivery Systems

In the recent decades, scientist and particularly those who work in the field of drug fabrication and pharmaceutical studies, start to recognize the need to discover new type of treatment methods for increasing number of diseases that have in respected number of cases moderate to low curative and recovery percentage after conventional oral or central medical administration, which has multiple drawbacks for both patient general condition an also the future survival and disease aggravation. Thus, we can notice the large number of studies that aim to
find out new methods to bring the drug in a quick simple and safe to the target site in the human bodies and achieve its mission directly. Drug Delivery Systems (DDS), that based mainly on a combination of pharmaceutical, polymer and bimolecular sciences, are considered one of the new promising and effective ways that give the opportunity to manage the pharmacodynamic, pharmacokinetic of the used Pharmaceutical substance, along with controlling the immunogenicity and host biorecognition to the drug molecules [112].

Indeed, DDS has showed an extraordinary capability in overcoming undesired obstacles that were preventing a number of conventional therapeutical methods to achieve big success against dangerous infections or life threatening diseases. Some of these problems as Allen et al. mentioned in a study published in Science journal in 2004 are discussed in the table.

There are different types of DDs such as Liposomes, Nano/Microsphere, Microemulsion, Nanosuspension, Micelles and Dendrimers. All of these new designed DDS have the ability to carry not just drug or medical molecules, but also it can successfully transport various types of protein hormones, miRNA, Enzymes to the host tissue and precisely the target cells.

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>EFFECTS OF DDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor solubility; since the hydrophobicity of the drug molecules prevents them to dissolve easily in aqueous mediums.</td>
<td>Using liposomes, for instance, as DDS that can offer hydrophobic and hydrophilic mediums that will improve rug solubility.</td>
</tr>
<tr>
<td>Toxicity of the drug to the surrounding tissue by the cause of extravasations phenomena</td>
<td>Control releasing of the drug directly to the target tissue can reduce toxic effects to the surrounding tissue.</td>
</tr>
<tr>
<td>Premature breakdown of the drug molecules after patient administration (effects of physiological PH)</td>
<td>Sustained release of the drug material from DDS which protect the with from rapid degradation will reduce the need of big doses.</td>
</tr>
<tr>
<td>Large body distribution leads to unwanted side effect to other organs.</td>
<td>DDS has a big ability to release the drug molecule locally and specifically to the targeted tissue, that lead to decrees distribution volume.</td>
</tr>
</tbody>
</table>
There are different types of DDs such as Liposomes, Nano/Microsphere, Microemulsion, Nanosuspension, Micelles and Dendrimers. All of these new designed DDS have the capability to carry not just drug or medical molecules, but also it can successfully transport various types of protein hormones miRNA Enzymes to the host tissue and precisely the target cells.

Recently, electrospinning technology ad attracted the attention of the DDS’s scientists and designer. They found that spun nanofiber can serve as a quite outstanding DDS via the large number potential properties those posses. For instance, electrospun nanofiber has a respected large surface area to volume ratio, this property can give apportioned to hold and release different kind of drugs and particularly those that have poor water dissolution, and subsequently low bioavailability after oral intake. Xie et al. reported the successful releasing of Paclitaxel (poorly water-soluble anticancer drug) that used for C6 glioma cells. The results shows a continues releasing of Paclitaxel from PLGA nanofiber mat for 60 days [113]. Furthermore, the new technical methods that discovered in terms of spun nanofiber formation have potential effects on both drugs carrying and delivering. Co-axial Electrospinning system has a very important application in the field of DDS, since it have the ability to produce what is called core/shell nanofiber that can hold and protect numerous easily degradable biological materials such as hormone, proteins and DNA molecules, thus provides an excellent and trustful way to ensure an intact transformation of easily denaturized materials [114].

Moreover, spun nanofiber has high flexible formulation methods, by which DDs
The designer can get advantage for fabrication various different nanofiber styles to be used in oral administration, topical cutaneous and subcutaneous application or even in terms of pulmonary administration, particularly by getting advantage of drug–polymer or drug-lipid complex that enhance the drug releasing rate and subsequent bioavailability or a great degree [114]. Yang et al. (2016) has reported in his study the formation Diclofenac sodium (DS) delivery system using novel tri-axial method for nanofiber spinning. He mentioned the successful production of DS-lipid (Lecithin)/polymer Eudragit S100 core/shell nanofiber that has the ability to protect the drug material from stomach acidity via the pH-sensitive layer of Eudragit S100 which approved to be dissolved in the neutral pH (PH around 7) , and gives subsequent advantage to release more drug material at the colonic lumen in which nanofiber structure will dissolve and giving the opportunity to form DS-lipid sub micro particles that lead to more prolonged releasing rate , along with increasing the permeability of DS through colonic lumen. [116].

In the same context, a number of studies reporting the high quality of drug encapsulation by nanofiber. these new era of encapsulation method can be a promising therapeutic way for number of diseases especially cancer diseases [40]. Also the high surface area of nanofiber has the ability not just to increase the encapsulation efficiency of drug molecules, but also play big roll for mass carrying and releasing of various drugs[114].

Relating to our study, and by getting advantage of the above facts, we used electrospun nanofibers as an effective DDS to carry two type of materials, a biologically easy degradable protein TGF-β, and also an herbal anticancer drug called Baicalein.

2.2.5.2.1. Baicalein

Baicalein is one of the ancient Chinese herbal medicine that used widely for the treatment of different disorders such as hypertension, atherosclerosis, common cold, and dysentery, hepatitis and hyperlipidemia [117] . This potential bioactive Flavonoid compound can be found mainly in the root of Scutellaria baicalensis, which grown in number of Asian countries particular China and South Korea.
Recently, a considerable number of studies have been carried out to investigate the curative effects that Baicalein probably possesses for various types of diseases, and nowadays we can find the large number of publications reporting the impacts of Baicalein as anti-d binding to hydroxyl molecules (shape), thus having the chemical formula as; 5, 6, 7-Trihydroxyflavone; (C\textsubscript{15}H\textsubscript{10}O\textsubscript{5}). Having molecular weight at about 270.24 g/mol with relatively high melting point at about 264 - 265 C.

This phynolic compound is believed to have effective impacts on different types of disease that till now the medical researchers and scientist couldn’t find a successful curative treatment for them like some cancer and inflammatory
diseases. The key role of baciacelin effect is its ability to make an extrinsic as well as intrinsic blocking to specific molecules during the disease pathway activation processes, and subsequently inhibited the expression of some proteins which considered being responsible for the

2.2.5.2.1.1. Inflammatory Diseases

It is believed that human body has defense system against any kind of damage that might happen to any part or organ called inflammation. The sign of inflammatory process can be easily noticed as acrudeness and congestion in the place of damaged tissue leading to blood high flow and subsequent curing in the injured sites. this simple phenomena, as we may see, is being induced and accurately controlled by hundreds of cytokines, receptor molecules, intercellular binding proteins and nucleus signal transduction molecules that arrange in different extracellular and intracellular signaling pathways. These incredible and high coordination reaction events, binding and dislodging, activation and inhibition processes aim to make the cell getting informed to any extrinsic change may happens and insure further appropriate respond to this changes.

PI3K/Akt/mTOR and PI3K/Akt/NF-kB (Phosphatidyl Inositol 3-kinase (PI3K), Protein kinase B (PKB), also known as (Akt), mammalian target of rapamycin (mTOR) and Nucleus Factor- kappa B protein ) signaling pathways are considered as most effective activation key process in different types of disease including inflammatory diseases and cancer. They provide transduction of any stimulation that comes to the cell and received by cell membrane receptors and carry it to the nucleus leading to further regulation genetic translation and protein expressions[122]. In most inflammatory diseases, NF-kB uncontrolled excessive activation believed to play a crucial roll in the development of such diseases. Therefore, scientist nowadays are looking for new molecules and compound that can cause inhibitor to NF-kB protein during the diseases pathway activation process that will subsquintlyt be a promising way to cure huge number of patients suffering from such diseases. [123]

In this context, the natural herbal chinese traditional medicine, Baicalein, starts to appear as one of chemical compound that may possess potential and novel therapeutic properties. number of studies had carried out in order to find out the way of how Baicalein can be effective against inflammatory diseases, and the
results of this studies confirmed by scientific evidences that Baicalein block the activation of NF-κB transduction protein and also play a key role in down regulation of multiple compounds such as 12-LOX, TNF-α, IL-17, VCAM-1, ROS and IgE that will cause a notably amelioration of disease course and even complete recovery.[124]

Figure 2.21. Mechanism Of Baicalein For Amelioration Of Different Inflammatory Diseases

Thus, Baicalein has be proved to cause a theraoutic impact in the uncontrolled inflammation process that considered the main cause of large number of diseases, some of them will be reviewed in table 2.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Mechanism of action</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Suppress IL-17 expression leading to stop inducing other cytokine and inflammatory mediators that evoke the inflammation process inside the synovial joint.</td>
<td>[125]</td>
</tr>
<tr>
<td>Rhinitis Allergy and Asthma</td>
<td>Inhibition of IκBα phosphrylatoin processes leading to suppress production of activated NF-kB that results in reducing human mast cell stimulation and subsequently alleviates the diseases inflammatory symptoms.</td>
<td>[126]</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>Inhibition of TGF-β/SMAD inflammatory pathway which has a key role in up regulation of miR-21, miR-29 and miR-155 responsible for collagen deposition in the lung tissue and fibroblast activation.</td>
<td>[127]</td>
</tr>
<tr>
<td>Food Allergy-Related Bowel Disorder</td>
<td>Ameliorate the symptoms of bowel allergy by induce differentiation and activation of regulatory T (Treg) cells to produce forkhead box protein P3 (Foxp3) that suppress the action of TH-1, TH-2 and TH-17.</td>
<td>[128]</td>
</tr>
<tr>
<td>Liver Fibrosis</td>
<td>Amelioration of inflammatory process through inhibition of stellate cells which believed to be responsible for the development of fibrosis in the liver infrastructure.</td>
<td>[129]</td>
</tr>
<tr>
<td>Chronic kidney Disease</td>
<td>Inactivation of NF-kB signaling pathway, leading to reduce expression of TNF-α, IL-6, TH-17 and other cytokines responsible for stimulation and continuation of inflammatory process in the nephritic tissue.</td>
<td>[130]</td>
</tr>
</tbody>
</table>

### 2.2.5.5.2.1.2. Cancer diseases

It's believe that Baicalein has really an outstanding effect on cancer diseases course.
It’s approved now that any suppressive action to one of the activation process throughout the disease signaling pathway may hold a great importance in terms of stop cancer development. therefore, scientist has carry out a lot of studies in order to know find out whether Baicalein could be the promising therapeutic agent that large number of cancer patients are waiting get advantage of its curative effects.
Table 3. The Effects of Baicalein on Number of Cancer Diseases

<table>
<thead>
<tr>
<th>Cancer</th>
<th>effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brest cancer</td>
<td>Suppression of mTOR/SGK1 pathway by increase DDIT4 expression. Lowering SATB1 (special AT-rich sequence binding protein 1) expression, that controls hundreds of oncogenic gens.</td>
<td>[131, 132]</td>
</tr>
<tr>
<td>Prostate cancer (PC)</td>
<td>Induce apoptosis in PC cell by Mcl – down regulation. Inhibition of Caveolin-1/AKT/mTOR pathway.</td>
<td>[133, 134]</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Down regulation of GADD45α (growth arrest and DNA-damage-inducible protein 45 alpha) leading to inhibit cancer cell proliferation</td>
<td>[135]</td>
</tr>
<tr>
<td>Small Cell Lung Cancer</td>
<td>The proliferation of Cancer cell was inhibited by increase expression of DDIT4 that lead to suppress mTOR pathway processes.</td>
<td>[136]</td>
</tr>
<tr>
<td>Non small cell lung cancer</td>
<td>Inhibits growth of cancer cell through lowering 12-LOX and VEGF expression.</td>
<td>[137]</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Decrease expression of Bcl-2.</td>
<td>[138]</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Decrease expression of Vascular Endothelial Growth Factor (VEGF), hypoxia-inducible factor 1α, Proto-oncogene c-Myc and NF-kB.</td>
<td>[139]</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Cancer cell growth inhibition by DNA Damage Induced Transcript 4 (DDIT4) up-regulation and suppression of mTOR pathway activity.</td>
<td>[118]</td>
</tr>
<tr>
<td>Colorectal cancer – collitis associated cancer</td>
<td>Peroxisome proliferators-activated receptors γ (PPARγ) suppress inflammatory genetic expression by interaction. With NF-kB, P50, P65 and other subunits.</td>
<td>[140]</td>
</tr>
<tr>
<td>Liver cell cancer</td>
<td>Induces apoptosis to the cancer cell via down regulation of Cyclin D1 and inhibition of PI3K/AKT signaling pathway.</td>
<td>[141]</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Induces apoptosis via lipoxygenase pathway inhibition and Mcl-1 decrease production.</td>
<td>[142]</td>
</tr>
</tbody>
</table>
### 2.2.5.2.1.3. Other Diseases

Table 4. Other Therapeutic Effects of Baicalein on Various Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Field of action</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic</td>
<td>Anti-<em>Pseudomonas aeruginosa</em> biofilm. Synergetic effect with ciprofloxacin against methicillin-resistant <em>Staphylococcus aureus</em> (MRSA).</td>
<td>[146]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[147]</td>
</tr>
<tr>
<td>antiviral</td>
<td>Promising treatment for H5N1 virus via its viruses neuraminidase inhibition effect and also immunomodulator action in the normal human tissue. Synergetic affects with Ribavirin against (H1N1) Influenza A virus. Powerful inhibitor of HIV virus reverse transcriptase</td>
<td>[148]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[149]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[150]</td>
</tr>
<tr>
<td>Carcinogenic diseases</td>
<td>Reduce cardiac fibrosis in hypertensive mouse and inhibited cardiac cell apoptosis. Protect cardiac contractility, promote vasoreactivity and blood hypertension through the reduction in Nitric Oxide production and suppress inflammatory response.</td>
<td>[151]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[152]</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>Improvement in the Parkinson diseases symptoms by inhibiting dopmain metabolism, induce neurogenesis and promote walking and locomotor attitudes.</td>
<td>[153]</td>
</tr>
</tbody>
</table>

Although Baicalein has not been yet earned any FDA approval to use it safely, many studies have mentioned that there are no evidence that Baicalein has direct
and cleared toxicity impacts on normal tissue and cell, beside its noticeable effect on neoplastic cancer cells [124]. As a result, many studies had carried out in ordered to find more predictable ways that give the ability to increases the both drug specificity and bioefficiency. For instance, Kulbacka et al. has successfully synthesized solid lipid nanoparticles (SLN) carrying Baicalein molecules as an anticancer agent. He reported an increase nanoparticles - reuptake by Loco colonic cancer cells especially after applying an electrical field plusses to the cells, that leaded to increases the cellular permeability to SLN, thus improve the toxical effects against cancer cells [154]. Another study has been done by Babu et al. that demonstrated the usage of Chitosan nanoparticles as a good drug delivery system in order to use their outstanding properties (such as sustained drug releasing and effective crossing to the biological border) in the context of anticancer agent delivering. Babu chose to use Baicalein as anticancer drug and load it on Chitosan nanoparticles. then he noticed the correspond topical effect of Baicalein loaded nanoparticles against MCF7 breast cancer cell-line [155].

Moreover, a few number of studies have indicated the ability to use the advantage of electeospun nanofibers as one to he most effective Drug delivery systems in terms of biosynthesized functional graft fabrication that can perform multiple biological and structural tasks, for example; Nirmala et al. reported the formation of Polyvinyl alcohol PVA / Baicalein nanofiber based scaffold that can be used as transplantable graft or even can be made to be like a membrane for protection against UV-sun light, and also as a sterile wound covering that resulted from its anti bacterial effects against E.coli and S. aureus organisms [156]. Depending on this study, we used the same blended mixture of PVA and Baicalein with another blended solution of HA/ PEO / TGF-beta to form hetergenous nanofiber based structure that contain two different types of polymers driven nanofibers. This process has been performed by getting advantage of both Electrospinning method and also spun nanofiber as it considered a highly effective drug delivery system (DDS) those posses’ potential advantages like having a large surface area with high drug carrying ability, biodegradability with sustained releasing of drug material inside the transplanted fibrous structure and other important properties.
2.2.5.2.2. TGF-β

Transforming Growth Factor beta or TGF-β, known as one of the cytokines that belongs to the Transforming growth factors super family which engaged in cell development regulation and differentiation. Actually, TGF beta has multiple isoforms; TGF-β1, TGF-β2, TGF-β3 and TGF-β4 that have multiple overlapping functions and effects. Although the three isoform are similar to each other, however each one of them found to be expressed in different site of body tissue, for example, about 85% of TGF-β1 used to found in bone tissue, while 50% of TGF-β2 can be found mainly in kidney. [157]. TGF-beta is forms believed to be big proteins molecules, consisting of 390 - 412 amino acid that form C-terminal segment which will be the mature TGF beta after got secreted from the cell, and two other segment N-terminal and latency associated peptide which play a key role in the TGF beta pre-mature state [158]

The mature TGF beta will get into subsequent activation cascade before becoming an active molecule that can react with TGF-β receptors expressed at the cell surface, leading to the activation of intercellular signaling and transcription factors that will interact directly with cell nucleus and genetic expression [159]

There are numerous important effects that TGF-β is responsible for such as regulation of cell development, proliferation and apoptosis. It can also involve in inflammatory response down regulation from continues uncontrolled cell proliferation. especially the blocking effect of cyclin / CDK complex that leads to inhibition of subsequent phosphorylation of Retinoblastoma protein [160]

Since the important role that TGF-β plays in the cellular regulation and development, large number of studies have carried out aiming to get the TGF-β engaged in the various tissue secretion into the scaffold porous micrometer spaces that will mimic the natural structure of ECM and improving the cellular development. to prove that, Lee ey al. (2017) reported the usage of poly(lactic-co-glycolic acid) (PLGA) nanofiber holding TGF-beta as a growth factor to fabricate a membrane like structure that can improve both the viability and blood perfusion through Skin Flap that used frequently in reconstruction operation for massive skin loss or facial skin damage. The end products showed cellular good activity in side
TGF beta holding scaffold, while in vivo testing on mouse, revealed a high blood perfusion with lower tissue necrosis in an implanted skin after 10 days of implantation [162].

Depending on the previous facts and information, we used the HA / PEO blended nanofibers as a carrier to hold TGF-beta protein molecules aiming to mimic the natural structural state of human extracellular matrix, that will provide a suitable medium for normal cellular growth and also cellular development inside the nanofiber structure.
3. MATERIALS AND METHODS

3.1. Chemical Materials and Apparatus

Hyaluronic acid (Sodium Hyluronate - HA) 11.000.000 g/mol, Italy - Polyethylene oxide (PEO), 600.000 g/mol, Sigma Aldrich, USA - Baicalein 270.24 g/mol Sigma Aldrich, China – TGF beta 2 human recombinant, 5UG, Sigma Aldrich, USA, Deionized Distilled Water in Hacettepe laboratories, Turkey.

Production of nanofiber was done by the usage of Electrospun device derived from 111T671 TÜBİTAK project. Assessment of nanofiber characterization was done by using Scanning Electron Microscope, SEM (FEI, Quanta 200F-Netherland) and also gold coating device (gatan, model 682) at UNAM, Bilkent University, Turkey. Fourier Transform Infrared - spectroscopy analysis and testing FT-IR done at Prof. Ismail boyatci’s Laboratory, Food engineering, Hacettepe University, Turkey.

3.2. Polymers Solutions Preperation

First we prepared the polymers solutions. In our study we used to polymers blended solution. The first solution contained HA blended with PEO containing TGF-β2. And the second solution contains PVA with Baicalein. HA / PEO / TGF-β2 solution was prepared by first adding of 0.1% of HA to 5ml deionized distilled water and stirring it for 15 minutes at about 29 C to ensure complete dissolution. then we added 10% PEO to the same solution and mixed it vigorously at temperature not more than 29 C till having a homogenously white turbid solution, than we let the solution to be stirred in room temperature for 24 hours on the mixer at about 150 mpr. After that we added 40ng/ml of pure nondiluted TGF-β and mix it for 10 minutes.

PVA / Baicalein solution was prepared by adding 7% PVA slowly to a cold 5 ml deionized distilled water and let it in the room temperature for 24 hours without stirring. Then we stirred it at about 65 C for 2hour to have a homogenous transparence solution. then we added different concentrations of Baicalein to PVA solution; 0.2%, 0.5%, 1%, 2% to four prepared 7% PVA solutions and mixed...
them gently at temperature of 73 °C for hours to ensure good dispersion of Baicalein molecules in the solution. Then we leave them to in the room temperature for 30 minutes.

### 3.3. Electrospun Nanofiber Production

In our study we use two types of polymers solution with mixing them in order to produce a homogeneous nanofiber bases scaffold containing two different kinds of nanofibers but using Electrospinning apparatus. For this purpose we filled two syringes with two different polymer solutions and perform the Electrospinning process alternatively by starting spinning with 0.4ml of (HA / PEO / TGF-β2) solution then putting PVA / Baicalein solution and spinned about 0.4ml of it. We repeated the same process till we spinned 2 ml of each solution. We use 5ml syringe with needle of 0.8 X 36 mm diameter X long and we sated the electrospinning parameter as we can see in table 5.

![Electrospinning Device](image)

**Figure 2.22. Electrospinning Device**
<table>
<thead>
<tr>
<th>POLYMERS</th>
<th>CONCENTRATION</th>
<th>PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tow syringe 1. PVA / Baicalein 2. PEO/HA/TGF-β</td>
<td>1. 7% / 0.2% 2. 10% / 0.1% / 40ng per ml</td>
<td>1- PVA / Baicalein Voltage: 15 KV TCD: 19 - 20 cm Pump: 0.016 µ/min 2- PEO/HA/TGF-β Voltage: 15 KV TCD: 20 cm Pump: 0.05 µ/min</td>
</tr>
<tr>
<td>1. Tow syringe 1. PVA / Baicalein 2. PEO/HA/TGF-β</td>
<td>1. 7% / 0.5% 2. 10% / 0.1% / 40ng per ml</td>
<td>1- PVA / Baicalein Voltage: 15 KV TCD: 17.3 cm Pump: 0.013 µ/min 2- PEO/HA/TGF-β Voltage: 15 KV TCD: 20 cm Pump: 0.05 µ/min</td>
</tr>
<tr>
<td>1. Tow syringe 1. PVA / Baicalein 2. PEO/HA/TGF-β</td>
<td>1. 7% / 1 % 2. 10% / 0.1% / 40ng per ml</td>
<td>1- PVA / Baicalein Voltage: 15 KV TCD: 17.3 cm Pump: 0.02 µ/min 2- PEO/HA/TGF-β Voltage: 15 KV TCD: 20 cm Pump: 0.05 µ/min</td>
</tr>
<tr>
<td>1. Tow syringe 1. PVA / Baicalein 2. PEO/HA/TGF-β</td>
<td>1. 7% / 2% 2. 10% / 0.1% / 40ng per ml</td>
<td>1- PVA / Baicalein Voltage: 15 KV TCD: 19.3 cm Pump: 0.02 µ/min 2- PEO/HA/TGF-β Voltage: 15 KV TCD: 20 cm Pump: 0.05 µ/min</td>
</tr>
</tbody>
</table>
3.4. Assessment Of Nanofiber Characterization

3.4.1. Scanning Electron Microscope

We prepared the sample by cutting the scaffold sample in dimension of 1cm X 0.8 cm and sited them on holders. After that, we performed 2-3 minute of gold coating process to ensure that our samples were suitable to be assessed by SEM device. Then we putted the gold coated sample inside SEM device in order to get detailed information about the surface morphology and characteristic shape of our produced nanofiber.

3.4.2. Fourier Transform Infrared - Spectroscopy Analysis

We used FT-IR device in order to chemical characterization of scaffold infrastructure, the presence of distinct materials and whether any of the used material has formed a new bonds with other scaffold components.
4. FINDINGS

4.1. Produced Nanofiber Based Scaffold Samples
As we see in figure 4.1 the end results of our produced sample were formed in a sheath shape with white yellowish color. The scaffolds possessed elastic properties that increase by increasing the concentration of added Baicalein.

![Figure 4.1](image)

Figure 4.23. Our produced PEO/HA/TGF-beta 2 + PVA/Baicalein Nanofiber Scaffold Samples With Different Baicalein Concentration(A1, A2) 0.2% Baicalein, (B1, B2) 0.5% Baicalein, (C1, C2) 1% Baicalein, (D1, D2) 2% Baicalein.

4.2. Characterization Of Nanofiber Scaffolds

4.2.1. Scanning Electron Microscope Results
In the table 6. (Below) we can find the spinning results of HA/ PEO with different concentration, PVA alone and PVA/ Baicalein with their SEM results.
Table 5 Review 1 Of Different Polymer Solutions Spinning findings That Used In Our Study, and The SEM Appearance Results For Each One of Them.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Polymers</th>
<th>Percentage</th>
<th>spinning</th>
<th>SEM appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>PEO/HA</td>
<td>1:1</td>
<td>NO</td>
<td>-</td>
</tr>
<tr>
<td>1%</td>
<td>PEO/HA</td>
<td>2:1</td>
<td>NO</td>
<td>-</td>
</tr>
<tr>
<td>2%</td>
<td>PEO/HA</td>
<td>3:2</td>
<td>YES</td>
<td>thin layer</td>
</tr>
<tr>
<td>4%</td>
<td>PEO/HA</td>
<td>7:3</td>
<td>YES</td>
<td>Few fiber formed</td>
</tr>
<tr>
<td>In 5 ml water</td>
<td>Gelatin/PEO/HA</td>
<td>4:2:1</td>
<td>YES</td>
<td>No fiber</td>
</tr>
<tr>
<td>In 5 ml water</td>
<td>Gelatin/PEO/HA</td>
<td>6:3:1</td>
<td>YES</td>
<td>No fiber</td>
</tr>
<tr>
<td>5%</td>
<td>PEO/HA</td>
<td>5:1</td>
<td>YES</td>
<td>Fiber formed , beads+</td>
</tr>
<tr>
<td>5%</td>
<td>PEO/HA</td>
<td>10:1</td>
<td>YES</td>
<td>Fiber formed, beads ++</td>
</tr>
</tbody>
</table>

Table 6 Review 2 Of Different Polymer Solutions Spinning findings That Used In Our Study, and The SEM Appearance Results For Each One of Them.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Polymers</th>
<th>Percentage</th>
<th>spinning</th>
<th>SEM appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>PEO</td>
<td>150 mg in 3 ml water</td>
<td>YES</td>
<td>No fiber</td>
</tr>
<tr>
<td>10% / 0.1%</td>
<td>PEO/HA</td>
<td>-</td>
<td>YES</td>
<td>Fiber (135 nm) , less beads</td>
</tr>
<tr>
<td>5% / 0.2%</td>
<td>PEO/HA</td>
<td>-</td>
<td>YES</td>
<td>Fiber formed , beads ++</td>
</tr>
<tr>
<td>10% / 0.1% / 40ng per ml</td>
<td>PEO/HA/TGF-β</td>
<td>-</td>
<td>YES</td>
<td>-</td>
</tr>
<tr>
<td>10% / 0.1% / 40ng per ml / 5mg</td>
<td>PEO/HA/TGFβ/Baicalein</td>
<td>-</td>
<td>YES</td>
<td>No SEM picture because of PEO and Baicalein oxidation.</td>
</tr>
<tr>
<td>7%</td>
<td>PVA</td>
<td>-</td>
<td>YES</td>
<td>Fiber formed (200-325 nm)</td>
</tr>
<tr>
<td>10%</td>
<td>PVA</td>
<td>-</td>
<td>YES</td>
<td>Fiber formed (250-400 nm)</td>
</tr>
</tbody>
</table>
Table 7. Review 3 Of Different Polymer Solutions Spinning findings That Used In Our Study, and The SEM Appearance Results For Each One of Them.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Polymers</th>
<th>spinning</th>
<th>SEM appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>13%</td>
<td>PVA</td>
<td>YES</td>
<td>Fiber formed (340-470 nm)</td>
</tr>
<tr>
<td>Tow syringe</td>
<td>PVA / Baicalein</td>
<td>YES</td>
<td>Fiber formed (85-140 nm)</td>
</tr>
<tr>
<td>1- 7% / 0.2%</td>
<td>2- 10% / 0.1% / 40ng per ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% / 0.1% / 40ng per ml</td>
<td>PEO/HA/TGF-β</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Review 4 Of Different Polymer Solutions Spinning findings That Used In Our Study, and The SEM Appearance Results For Each One of Them.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Polymers</th>
<th>spinning</th>
<th>SEM appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tow syringe</td>
<td>PVA / Baicalein</td>
<td>YES</td>
<td>Fiber formed (103-165 nm)</td>
</tr>
<tr>
<td>1- 7% / 1%</td>
<td>2- 10% / 0.1% / 40ng per ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>PEO/HA/TGF-β</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(As we can see in figure 4,2 , 4,3 and 4,4), the shape of nanofiber as well as formation of beads has changed clearly from a high beaded nanofibers in 5% (PEO/HA 10/1) and (PEO/HA 5/1) , to have good shaped. Continues lined, nonwoven nanofiber (81nm -130nm) with almost no beads or very small number of scattered beads in PEO/HA 10%wt /0.1%wt scaffold nanofibers.

Also we found that changing in the PVA solutions concentration from 13%wt up to
7% wt will result in decreasing the average diameter of produced nanofiber. Which measured about 410 nm in PVA 13% wt to be about 200 nm in PVA 7% wt nanofibers (figure 4.5).

Depending on the previous results we chose to use PEO/HA/TGF-beta2 (10% wt/0.1%/ 40ng / ml) to spin it with the same time with PVA 7% by using two different syringes for each of the two solutions. And we added Baicalein to PVA solution and spun them with different concentration of Baicalein; 0%, 0.2%, 0.5%, 1% and 2%. And we got SEM images for all samples .see figure 4.6, 4.7, 4.8, 4.9, 4.10 respectively. Also we take a SEM image for scaffold containing 0.2% after 27 day of production and we noticed the degradation sign that happened to the fibers.

The average diameter of produced nanofiber was clearly increased with increasing Baicalein concentration as it started with an average of 1120 nm for 0.2% Baicalein scaffold, then 270 nm for 0.5% wt Baicalein scaffold. And reach 280 nm for the last used concentration of Baicalin of 2% wt.( figure 4.11, 4.12, 4.13, 4.14 and 4.15.)
Figure 4.24. SEM Images of Electrospun PEO/HA 5%wt Nanofibers; (a) PEO/HA 10/1, (b) PEO/HA 5/1 show high level of beads formation.
Figure 4.25. SEM Image of Electrospun PEO/HA (5%/2%) Nanofibers; (a) 10,000 X magnification, (b) 40,000 X magnification show medium level of beads formation.
Figure 4.26. SEM Image of Electrospun PEO/HA (10%/ 0.1%) Nanofibers (a) 10.000 X magnification, (b) 10.000 X magnification and (c) 40.000 magnification show disappearing of beads within the produced nanofibers.
Figure 4.27. SEM Image of Electrospun PVA Nanofibers in 50.000X (a) 13%wt PVA, (b) 10%wt PVA and (c) 7%wt PVA Show Different Nanofiber Diameters
Figure 4.28. SEM Image Of Electrospun PEO/HA/TGF-Beta2 With PVA / 0% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.
Figure 4.29. SEM Image Of Electrospen PEO/HA/TGF-Beta2 With PVA / 0.2% Baicalein Nanofibers; (A) 1,000 X Magnification, (B) 20,000 X Magnification And (C) 50,000 X Magnification With Fibers Diameter In Nanometer. Also The Three Image Clearly Show Degradation Happened To The Nanofibers After 3 Weeks Of Production.
Figure 4.30. SEM Image Of Electrospun PEO/HA/TGF-Beta2 With PVA / 0.5% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.
Figure 4.31. SEM Image Of Electrospun PEO/HA/TGF-Beta2 With PVA / 1% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.
Figure 4.32. SEM Image Of Electrospun PEO/HA/TGF-Beta2 With PVA / 2% Baicalein Nanofibers; (A) 1.000 X Magnification, (B) 20.000 X Magnification And (C) 50.000 X Magnification With Fibers Diameter In Nanometer.
Figure 4.33. Distribution Of Nanofiber Diameters Measurements For PEO/HA/TGF-Beta2 With PVA / 0% Baicalein Nanofibers.

Figure 4.34. Distribution Of Nanofiber Diameters Measurements For PEO/HA/TGF-Beta2 With PVA / 0.2% Baicalein Nanofibers.
Figure 4.35. Distribution Of Nanofiber Diameters Measurements For PEO/HA/TGF-Beta2 With PVA / 0.5% Baicalein Nanofibers.
Figure 4.36. Distribution Of Nanofiber Diameters Measurements For PEO/HA/TGF-Beta2 With PVA / 1% Baicalein Nanofibers.

Figure 4.37. Distribution Of Nanofiber Diameters Measurements For PEO/HA/TGF-Beta2 With PVA / 2% Baicalein Nanofibers.
4.2.2. FT-IR Findings

As we can notice from figure 4.16, Infrared absorbance bands characteristic for HA were observed at 3290 cm$^{-1}$, 1602 cm$^{-1}$ and 1034 cm$^{-1}$. These values correlated with those listed for carbonyl (C=O), and hydroxyl (O-H) and amine (N-H) moieties, respectively [76]. absorbance bands for PEO were noticed at 2889 cm$^{-1}$ and the strong triplet band at 1147 cm$^{-1}$, 1104 cm$^{-1}$ and 1064 cm$^{-1}$ with a maximum at 1090 cm$^{-1}$ are attributed to the C-H stretch and C-O-C accordingly of the PEO [163]. PVA characteristic absorbent peaks can be observed at 3300 cm$^{-1}$, 2900 cm$^{-1}$,1400 cm$^{-1}$ and 1088 cm$^{-1}$ which contribute with O-H, C-H, CH-OH and C-O groups respectively [164].

As we see in figure 4.17, Baicalein absorbs bands were noticed at 3400 cm$^{-1}$, 1655 cm$^{-1}$,1614 cm$^{-1}$, 1470 cm$^{-1}$, 1292 cm$^{-1}$, 1159 cm$^{-1}$, 1083 cm$^{-1}$, 880 cm$^{-1}$, 825 cm$^{-1}$, 637 cm$^{-1}$ and 467 cm$^{-1}$. Which correlate with O-H stretching, C=C stretching, CCO bending, HCC bending (from 1292 cm$^{-1}$ to 1083 cm$^{-1}$) , CCO, HCCC torsion and CCC bending (637 cm$^{-1}$ to 426 cm$^{-1}$) ,respectively [165].

Infrared spectrum of our produced PEO/HA/TGF-beta2 with PVA / Baicalein nanofiber scaffold included the characteristic absorbent bands for HA ( OH at 3328 cm$^{-1}$, CO at 1600 cm$^{-1}$ and NH at 1044 cm$^{-1}$ see figure 4.21) . PEO was also included with its characteristic bands spectrum at 2880 for CH stretching and 1100 for COC . PVA with its absorbent bands was observed at 3312 cm$^{-1}$, 2940 cm$^{-1}$ 1428 cm$^{-1}$ and 1089 cm$^{-1}$ ,see figure 4.21. These characteristic bands of HA, PEO and PVA were observed in all scaffolds samples, which means that these material were present within the chemical composition of our produced scaffolds.

Characteristic absorbent bands intensity for Baicalein was ( at 3310 cm$^{-1}$for OH group stretching , and also at 610 cm$^{-1}$ for OCC bending) increased gradually with increasing in the Baicalein concentration in the scaffold samples , figure 4.19, 4.20, 4.21, and 4.22. This means that FT-IR spectrum results were correlated with chemicals compositions and their concentrations differences in within our produce nanofiber scaffold samples.
Figure 4.38. FT-IR Spectrum for PEO, HA and PVA
Figure 4.39. FT-IR Spectrum for Baicalein
Figure 4.40. FT-IR Spectrum for PEO/HA/TGF-beta2 (above), and PVA/Baicalein (below)
Figure 4.41. FT-IR Spectrum for PEO/HA/TGF-beta2 - PVA /0% Baicalein
Figure 4.42. FT-IR Spectrum for PEO/HA/TGF-beta2 - PVA /0.2% Baicalein

Figure 4.43. FT-IR Spectrum for PEO/HA/TGF-beta2 - PVA /0.5% Baicalein
Figure 4.44. FT-IR Spectrum for PEO/HA/TGF-beta2 - PVA /1% Baicalein
Figure 4.45. FT-IR Spectrum for PEO/HA/TGF-beta2 - PVA /2% Baicalein
Figure 4.46. FT-IR Spectrum for PEO / HA / Baicalein
5. RESULTS AND DISCUSSION

5.1. Polymer Solution Preparation

During polymer solution preparation we faced some problems, particularly when we let the solution to be stirred for 24 hours. That cause variant amount of water to evaporate, thus raising the concentration of our used polymer. To solve this problem we took a measurement of the solution weight before stirring it, and took another measurements to the weight of same solution after the end of stirring. Then we added the amount of water that lost during stirring.

It's good to mention that we did a trial to blend Baicalein with PEO/HA solution. Basically, by adding Baicalein to the clear noncolor PEO/HA solution, the color will change to be light yellow then it will be more darkly by continues mixing and heating to the solution. But after 15 minutes of adding Baicalein, we found that the color started to be change to black with appearing of black particles within the solution. This case didn't happened when we added Baicalein to PVA solution, in which the color remain yellow for more than 48 hours without appearing of any new particles within the solution. This phenomena may as a results of strong oxidation reactions that start one we added Baicalein to PEO/HA solution. Although the presence of HA which considered an antioxidant agent, however the amount of HA used in during solution preparation was very low (5 mg in 5ml DW). This thing made us to use PVA during spinning processing order not to affect the chemical nature of the Baicalein molecules.

5.2. Electrospinning And Nanofiber Production

Electrospinning parameters are believed to be a very important factor that affects the shape and appearance of Nanofibers. During our study, we tried to make a precise parametric optimization to each one of the spun polymer solutions. However, we found some difficulties to ensure a full control on the temperature and humidity parameter that might sometimes affect the nanofiber formation, and furthermore, affect the diameter of the produced nanofiber.

The way we used to perform the Electrospinning, in which we spun 0.4ml of one solution, then we changed the syringe and spun 0.4ml from another solution, although it provide a good opportunity to have a homogenous nanofiber structure.
in one scaffold sample, it may affect characterization test like SEM images and FT-IR analysis test, in which we depend on the surface layers of scaffold sample to assess the hole properties. This surfaces layer may be differ from sample to another as a results of the last polymer solution spun during nanofiber formation, or the sample that taken from the collector contain more fiber from one solution and lower fiber from another solution. During sample taking we tried to choose the best place of collector on which the two type of fibers has collected.

5.3. Assessment of Nanofiber Characterization

5.3.1. SEM Images

As we see from the SEM images, the concentration of polymer in the Electrospinning solution has a great effect on the shape of nanofiber, average diameter and even the formation of beads. The lower polymers concentration found to be more liable to form beads during spinning process that is because the electrical field is facing low viscosity and so low resistance in the time of jet initiation. This makes more amount of solution to erupt from Tailor cone and will be collected as beads with different diameters (figure 4.2, 4.3 and 4.4).

The scaffold sample with 0.2% et Baicalin was assessed under SEM after 21 days of production (figure 4.7), and the sign id destructed nanofibers is a good evidence on the degradation ability that our used polymer material posses, which ensure releasing of the functional molecule such h TGF-beta and Baicalein in sustained way to the nearby tissue.

The average diameter nanofiber found to increase gradually in correlation with Baicalein amount that has been added to the solution. But we can found that 1%wt Baicalein including nanofiber (figure 4.14) has a relatively thinner average diameter from the nanofiber containing 0.5%wt. This may be a results of some parameter that we couldn’t ensure full controlling on them like temperature and humidity that can affect the diameter of nanofiber for big degree.

5.3.2. FT-IR Analysis Spectrum

An overall observation to the results of IR spectrums revealed that the intensity of OH stretching and CCC bending absorbent bands at 3320 cm\(^{-1}\) and 620 cm\(^{-1}\) have increased with correlation with Baicalein concentration. The other groups that appeared in the Baicalein IR spectrum were not formed clearly on the IR spectrum.
of our produced scaffold samples. That is because spectrum of PVA, PEO, and HA may mask the appearance of different groups or Baicalein molecule had lose some bond in order to make another bonds with PVA molecules.

1%wt Baicalein scaffold sample did not show a great increasing in the intensity of OH and CCC absorbent band in comparing with other samples. However the test show the presence of both OH stretching at $3320\text{ cm}^{-1}$ and CCC bending at $608\text{ cm}^{-1}$ which characteristic for Baicalein bands spectrum. The intensity of another group like NH at $1090\text{ cm}^{-1}$ and CH2 at $2883\text{ cm}^{-1}$ which characteristic for HA and PEO respectively, have appeared strongly (figure 4.22). That happened as a result of being the surface layer of scaffold sample containing more amount of PEO/HA nanofibers which may mask the appearance of characteristic absorbent bands for Baicalein molecules.

The IR spectrum that taken for PEO/ Baicalein solution is strongly reflects the degree of distraction that happened to the chemical structure of Baicalein molecules. We notice that a strong band has formed at $3323\text{ cm}^{-1}$ and lower band intensity at $1639\text{ cm}^{-1}$ and $1081\text{ cm}^{-1}$ which correlate with OH stretching, C=O and CC, with disappearing of other characteristic Baicalein band like CCC bending at $625\text{ cm}^{-1}$.

As a result, we successfully produced HA / PEO / TGF- beta2 with PVA nanofiber scaffolds containing different concentration of Baicalein by getting advantage of the technology of Electrospinning to synthesis nanofibers with a diameter of nanometer scale. Furthermore we assessed the characterization of our produced nanofiber via studying of SEM images and also the analysis results of FT-IR spectrooscope which gives a clear idea about the chemical composition of the all produced scaffold samples.
6. REFERENCES


Conference Record of the 1993 IEEE. 1993. IEEE.


[34] Gupta, P., et al., *Electrospinning of linear homopolymers of poly (methyl


Kamprad, I. and K.-U. Goss, *Systematic investigation of the sorption


[96] Black, J., 1988 Western winter workshop on Tissue engineering:


Saito, M., T. Sasaki, and H. Matsuoka, *Vitamin B12 promotes Cx40 and


[115] Huang, J. and T. You, Electrospun nanofibers: from rational design, fabrication to electrochemical sensing applications, in Advances in Nanofibers. 2013, InTech.


Jung, S.Y., et al., *Baicalein Induces CD4⁺ Foxp3⁺ Tcells and Enhances
Intestinal Barrier Function in a Mouse Model of Food Allergy., 2016: p. 470-470.


[140] He, K., et al., *Baicalin and Ly294002 induces liver cancer cells apoptosis via regulating phosphatidyl inositol 3-kinase/Akt signaling pathway*. **2017**.


[145] Rajkumari, J., et al., *Facile green synthesis of baicalein fabricated gold nanoparticles and their antibiofilm activity against Pseudomonas aeruginosa PAO1*. Microbial Pathogenesis, **2017**.


CURRICULUM VITAE

Credentials

Name, Surname: Kamel BACHIMAM
Place of Birth: Damascus/Syria
Marital Status: Single
E-mail: k.bashimam@gmail.com
Address: Sogutozu Mah. Ankara\ Turkey

Education

BSc. : School of Medicine (MD), Syrian University for Science and Technology.
MSc. : Nanotechnology and Nanomedicine, Graduate Scholl for Science and Engineering, Hacettepe University .

Foreign Languages

English (advanced)
Turkish (Good)
Germany (preliminary)

Work Experience

Resident in the Internal Medicine Department - The Red Crescent Hospital in Damascus, Syrian Ministry of Health.
Resident in the Internal Medicine Department - Al Shifa Hospital. Damascus , Syria.

Areas of Experiences
Research Assistant at Hacettepe University. Using Electrospinning apparatus in order to produce a synthetic scaffolds from different types of polymeric nanofibers.

Research Assistant at Gazi University. Detection of Phenylalanine blood concentration by Raman spectroscopy using Silver Nanoparticles

Advanced Life Support Certificate - The European Resuscitation Council

Projects and Budgets
• HACETTEPE BAP: Production And Characterization Of TGF-β And Baicalein Containing Hyaluronic Acid Nanofibers By Using Electrospinning Method (18.900 TL)

Publication
- 
Oral and Poster Presentation
-
HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
THESIS ORIGINALITY REPORT

Date: 14/06/2017

Thesis Title / Topic: Production and Characterization of Hyaluronic Acid Nanofibers Containing TGF-β and Baicalein by Using Electrosprining Method

According to the originality report obtained by my thesis advisor by using the Turnitin plagiarism detection software and by applying the filtering options stated below on 14/06/2017 for the total of 98 pages including the a) Title Page, b) Introduction, c) Main Chapters, d) Conclusion sections of my thesis entitled as above, the similarity index of my thesis is 9 %.

Filtering options applied:
1. Bibliography/Works Cited excluded
2. Quotes excluded
3. Match size up to 5 words excluded

I declare that I have carefully read Hacettepe University Graduate School of Science and Engineering Guidelines for Obtaining and Using Thesis Originality Reports; that according to the maximum similarity index values specified in the Guidelines, my thesis does not include any form of plagiarism; that in any future detection of possible infringement of the regulations I accept all legal responsibility; and that all the information I have provided is correct to the best of my knowledge.

I respectfully submit this for approval.

Name Surname: Kamel Bachimam
Student No: N13125688
Department: Nanotechnology and Nanomedicine
Program: -
Status: ☒ Masters □ Ph.D. □ Integrated Ph.D.

ADVISOR APPROVAL

[Signature]
Title, Name Surname, Signature