Small Molecules: Application in Tissue Engineering

Presenters: Mahroo Hajimehdi, Nafiseh Pakdin, Mahya Sharifi, Fateme Ardehali
Supervisor: Dr. Bakhshandeh

Department of Biology, University of Tehran
Fall 2021

CONTENTS

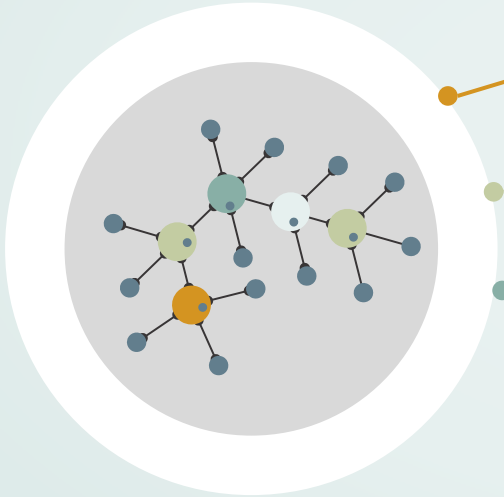
Introduction

What are Small Molecules?
Why are they important?

Small molecules are molecules with a molecular weight of < 1000 Da.

They are reproducibly synthesized through chemical reactions or extracted from organisms including plants.

EXAMPLES



Pharmaceutical Drugs

insulin, aspirin, antihistamines

Biological Molecules

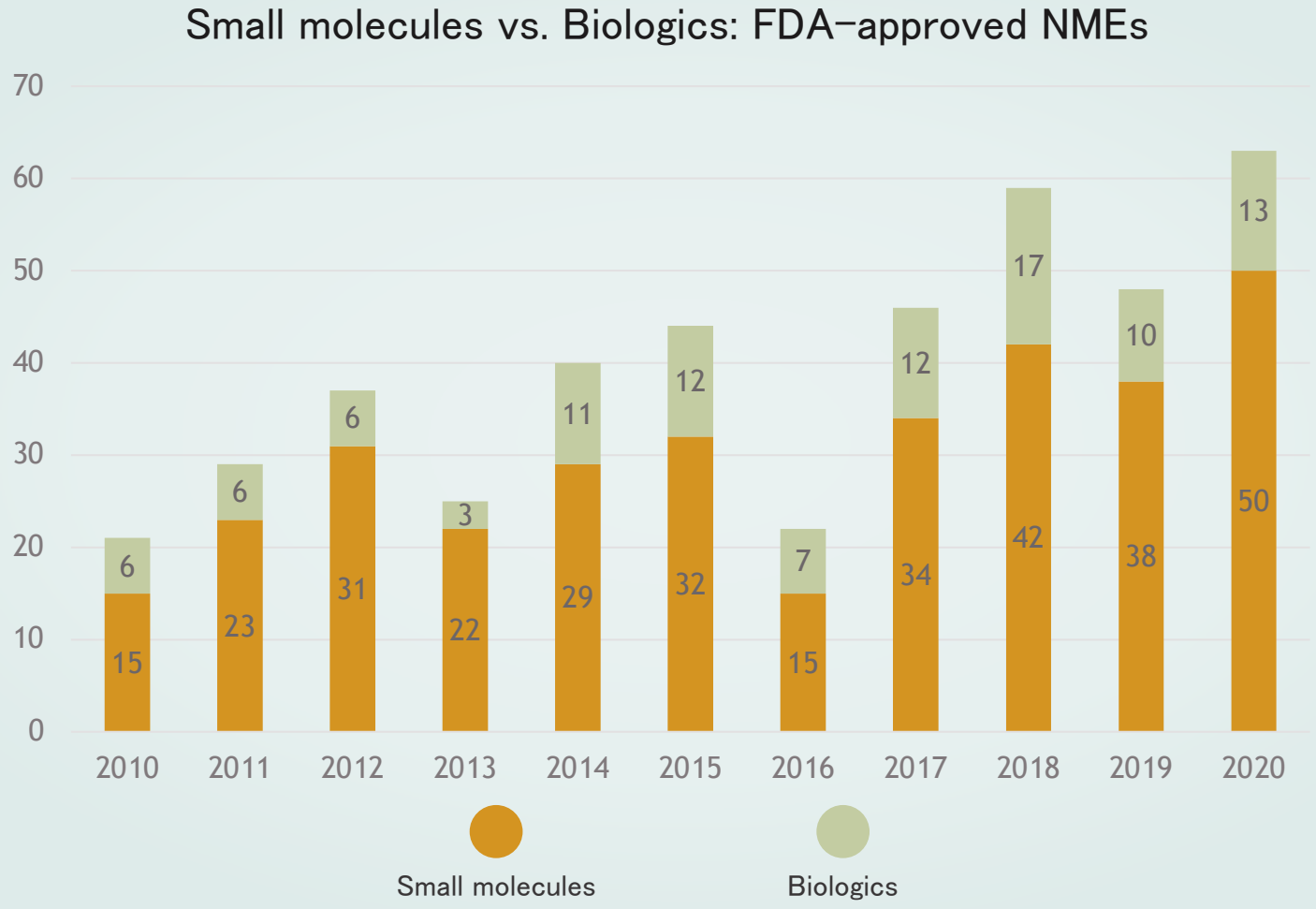
fatty acids, glucose, amino acids, cholesterol

Secondary Metabolites

glycosides, alkaloids, natural phenols

Do NOT Include

polysaccharides, proteins, nucleic acids


Small molecules vs. Biologics: FDA-approved NMEs
70
60
50
40
30
20
10
0
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
15
6
23
6
31
6
22
3
29
11
32
12
15
7
34
12
42
17
38
10
50
13
Small molecules
Biologics

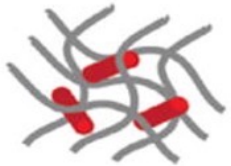
	Small Molecules	Biologics
Size	< 1 kDa	> 1 kDa
Stability	Chemically and thermally stable	Sensitive to heat, easily degraded
Polarity	Wide range	Polar
Cell Permeability	Extra & intracellular components, CNS	Extracellular
Delivery	Oral	Invasive
Drug-Drug Interaction	Prone	Less prone
Selectivity	Low	High
Prize	Low	High

A DISADVANTAGE

Small molecules use is limited by their nonspecific adverse effects on nontarget tissues and organs.

The key to success with utilizing small molecules is designing suitable delivery systems to localize and sustain the controlled release of small molecules to target sites.

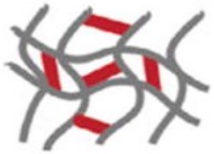
Entrapment



Covalent Binding

Adsorption

Crosslinking



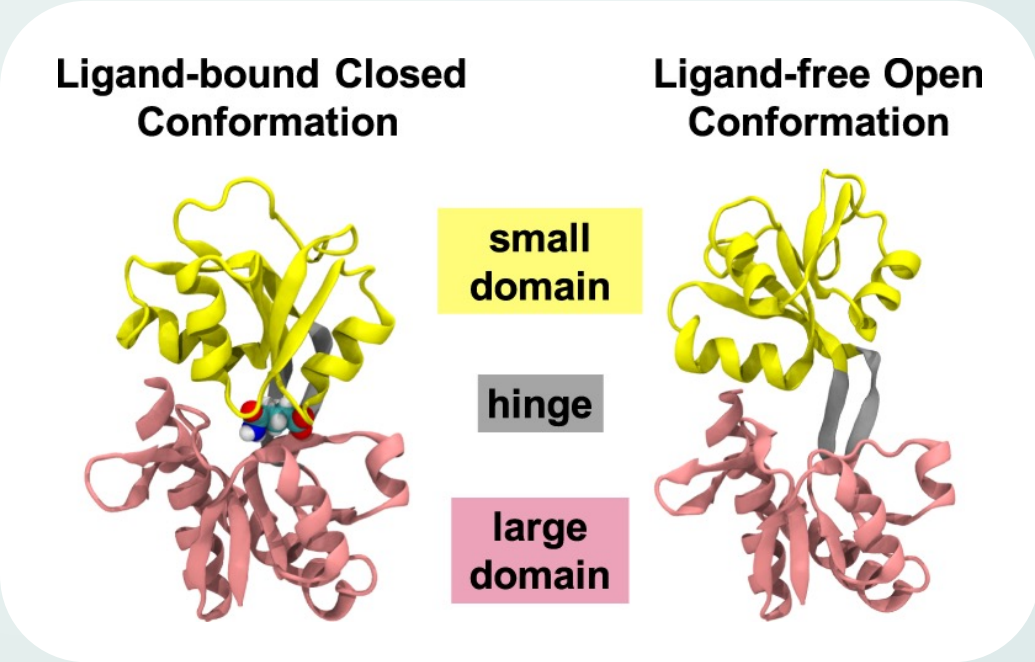
There are several types of incorporation technique. Each method has inherent advantageous depending on:

- The biomolecule chemical structure
- Intended scaffold fabrication method
- Desired drug release profile

Mechanism

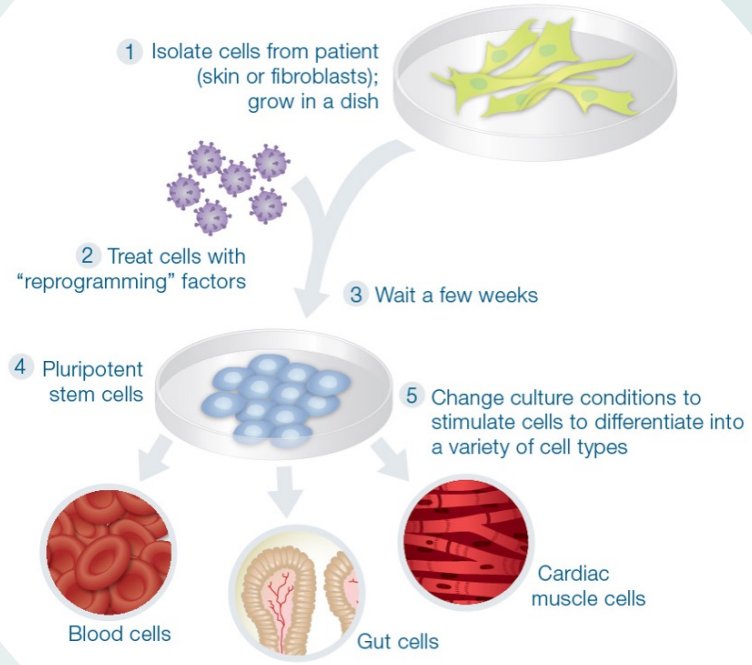
How do they effect their target
What aspects are of interest?

Small molecules can regulate the function of proteins as they bind to proteins to form protein–ligand complexes and induce conformational changes of the proteins at the same time.



Small molecules can also induce differentiation. **In tissue engineering**, the regenerative properties demonstrated by some small molecules have shown promise in retaining the functional and physical traits of natural tissues.

Small molecules have been used to generate iPSCs by acting as substitutes for genetic reprogramming factors.



The Small molecule compounds used in reprogramming protocols can be categorized into:

01

Epigenetic Modulators

02

Signaling pathways regulators (inhibitor/activator)

03

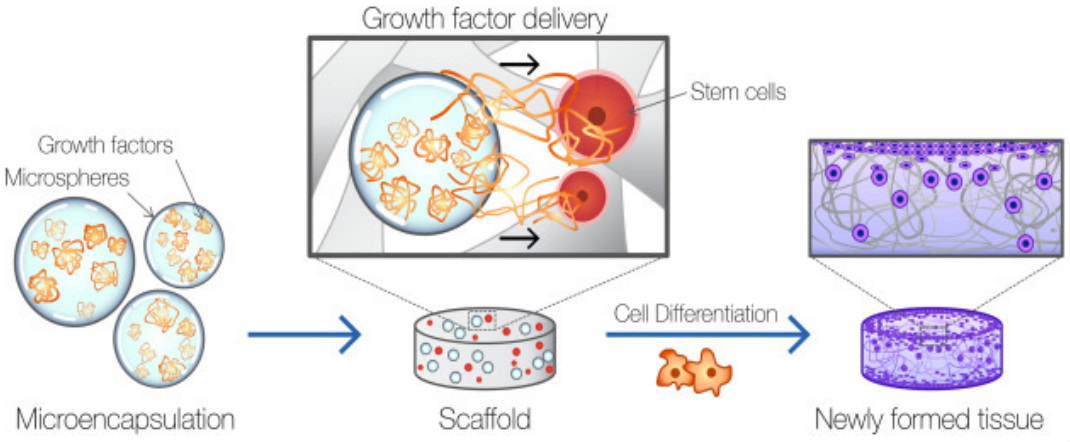
Metabolic regulators

04

Other factors that induce the characteristics of the designed cell type

Growth Factor

Growth factors are routinely used in the tissue engineering approach to expedite the process of regeneration.
GFs can upregulate or down-regulate cellular activities (adhesion, proliferation and differentiation)



Growth Factors

- Only few studies proceeded to clinical trials
- Low half-life
- Instability
- High cost
- Possible negative long term side effects
- Limited in certain scaffold-based applications requiring thermal processing, sterilization or prolonged exposure to solvents

Small Molecules

- Easier to manufacture or are available from bioresources
- Less costly
- Less prone to denaturation bioactivity does not depend on their higher order structure
- Unlikely to induce an immune response in the host easier cellular penetration

Application

Where is it used?
What are the effects?

Small molecule mediated bone formation

Small molecule mediated cartilage formation

Small molecule mediated neural regeneration

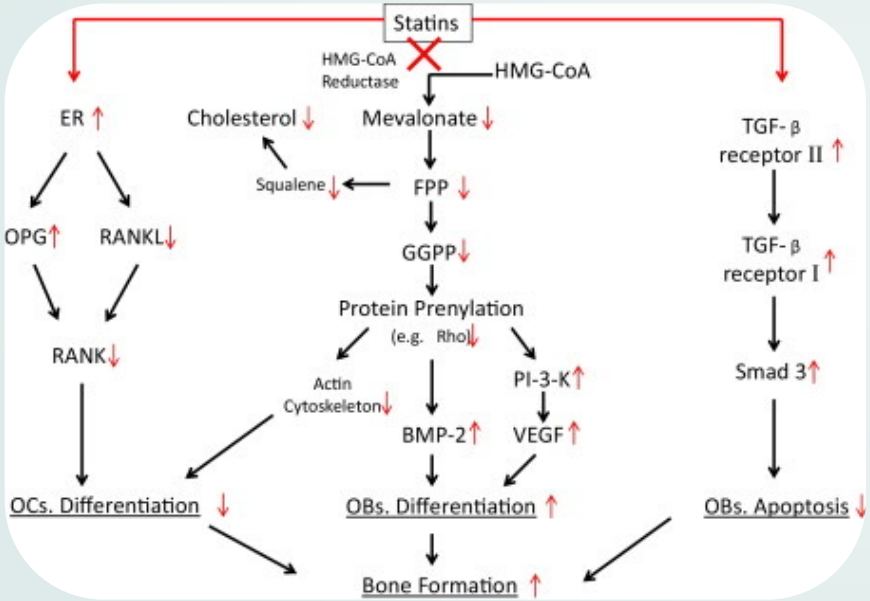
Small molecule mediated angiogenesis

Small molecule mediated muscle regeneration

Simvastatin

BMP

Bone Formation



Simvastatin primarily used for the management of hyper–cholesterolaemia, is a promising scaffold additive in bone regeneration.

- In its cholesterol lowering activity, it inhibits the enzyme HMG–CoA reductase by binding to it.
- In its bone regeneration activity, the same chemical moiety inhibit the formation and activity of osteoclast.

SVAK-12

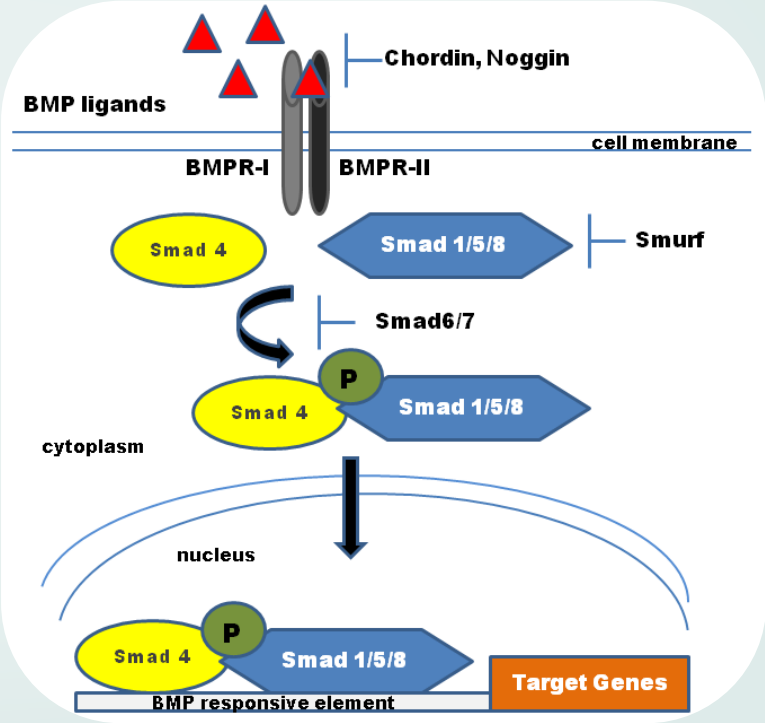
BMP

Bone Formation

SVAK-12 was identified as a novel BMP signaling activator for potentiating BMP-2 induced trans-differentiation of muscle cells into osteogenic lineages by interrupting the function of Smurf1.

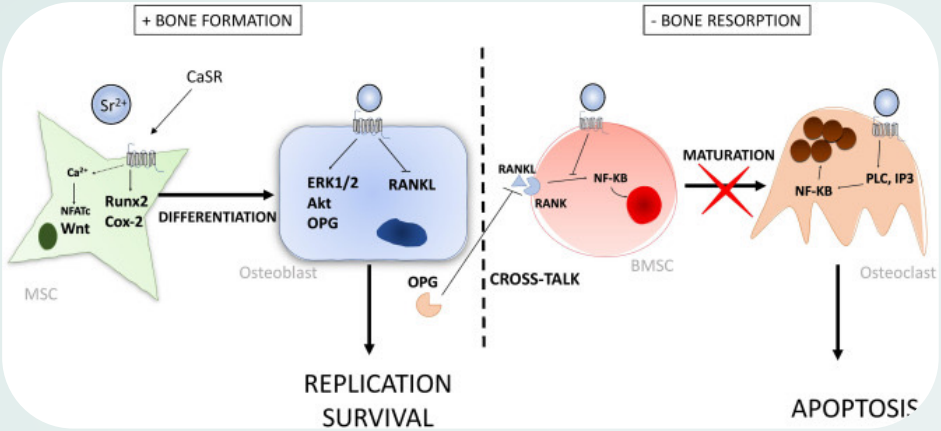
A single-dose of SVAK-12 (100, 250, or 500 μg) combined with a low-dose of exogenous BMP-2 (1.5 μg) enhanced ectopic bone formation when both therapeutics were subcutaneously implanted in rats.

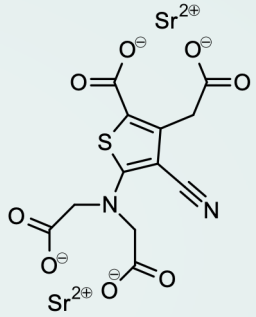
.



Strontium ranelate

Cartilage Formation

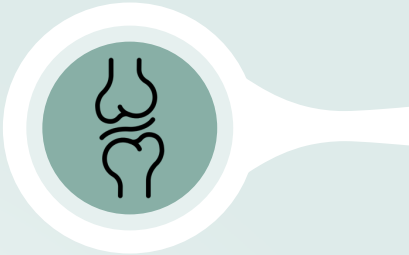




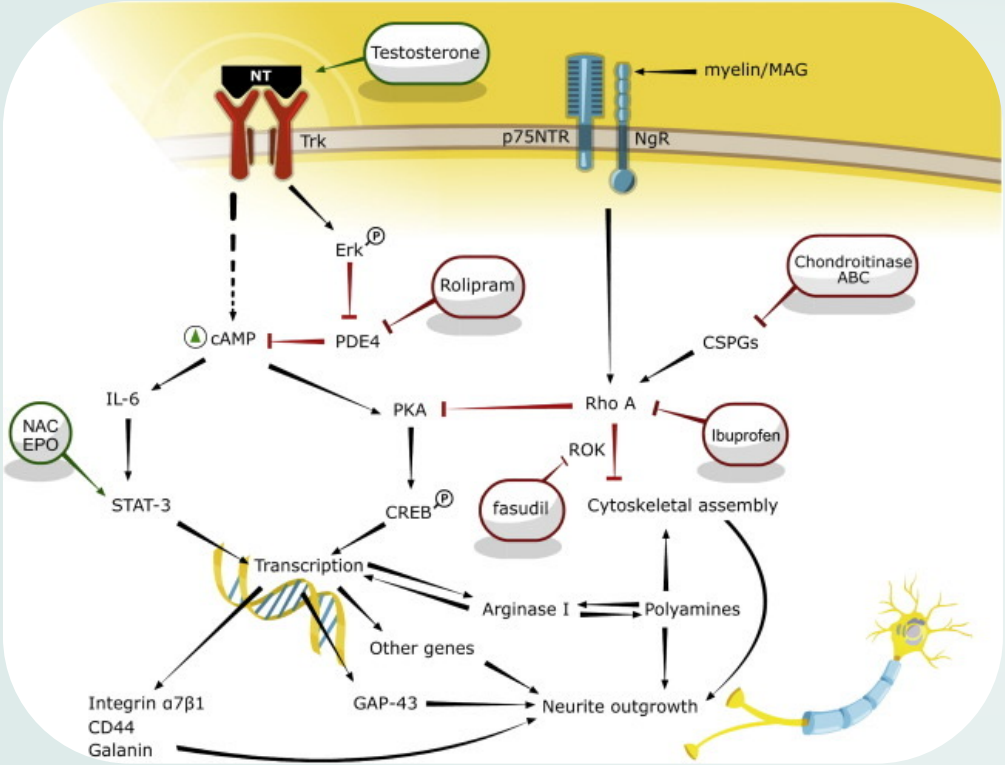
- Is a strontium (II) salt of ranelic acid which acts as an antiosteoporotic agent which both increases bone formation and inhibits bone resorption.
- Sr treatment upregulates osteogenic gene expression in osteoblasts, which has been found to be dependent on mitogen–activated protein kinase (MAPK) signalling and ERK 1/2 phosphorylation.
- Sr treatment has been shown to decrease osteo–clastogenesis and therefore reduce the number of mature osteoclasts in vitro, which is directly related to a reduction in the formation of resorption pits.

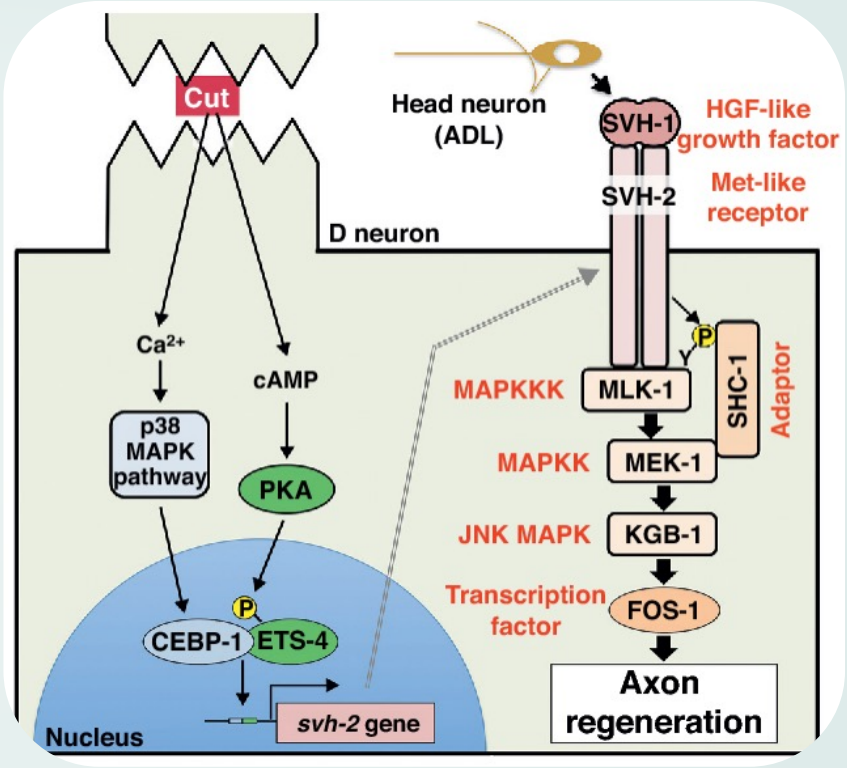
db-cAMP

Neural Regeneration



- Dibutyryl cyclic-AMP (db-cAMP) has a significant effect on neural regeneration.
- Exogenous administration of db-cAMP into the injured area significantly improved motor recovery in a rat ischemia model. The db-cAMP treated rat groups showed increased expression of growth associated protein 43 (GAP-43), a protein associated with axon regeneration and neural regeneration.
- Treatment with db-cAMP effectively induces neuronal differentiation of neural stem/progenitor cells (NSPCs), resulting in increase of cells expressing beta III tubulin, a neural precursor protein.

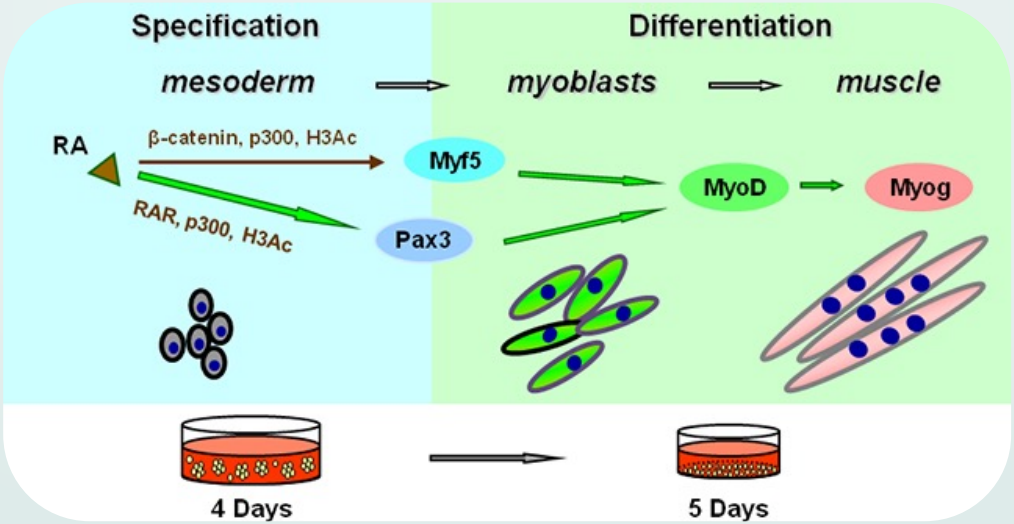

Testosterone
NT
Trk
p75NTR
NgR
myelin/MAG
Erk
P
Rolipram
Chondroitinase ABC
cAMP
PDE4
CSPGs
IL-6
PKA
Rho A
NAC
EPO
Ibuprofen
ROK
STAT-3
CREB
fasudil
Cytoskeletal assembly
Transcription
Arginase I
Polyamines
Other genes
Integrin α7β1
CD44
Galanin
GAP-43
Neurite outgrowth


Cut
Head neuron
(ADL)
SVH-1
HGF-like
growth factor
SVH-2
Met-like
receptor
D neuron
Ca2+
cAMP
p38
MAPK
pathway
PKA
P
CEBP-1
ETS-4
svh-2 gene
Nucleus
MAPKKK
MLK-1
SHC-1
Adaptor
MAPKK
MEK-1
JNK MAPK
KGB-1
Transcription
factor
FOS-1
Axon
regeneration

Retinoic Acid

Muscle Regeneration

- Small molecules can induce stem cells to differentiate into a myoblastic lineage.
- Embryonic stem cells treated with low-level doses of retinoic acid showed increased expression of the myoblastic markers (Pax3, Meox1 and myogenin) over control, which suggests the formation of myoblast progenitor cells (MPC), which ultimately differentiate into myoblasts.

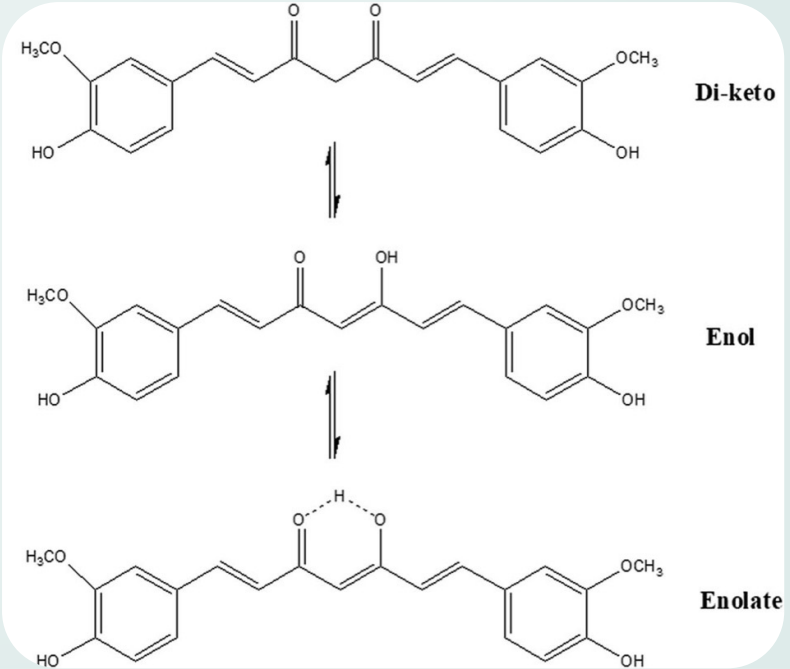


Curcumin

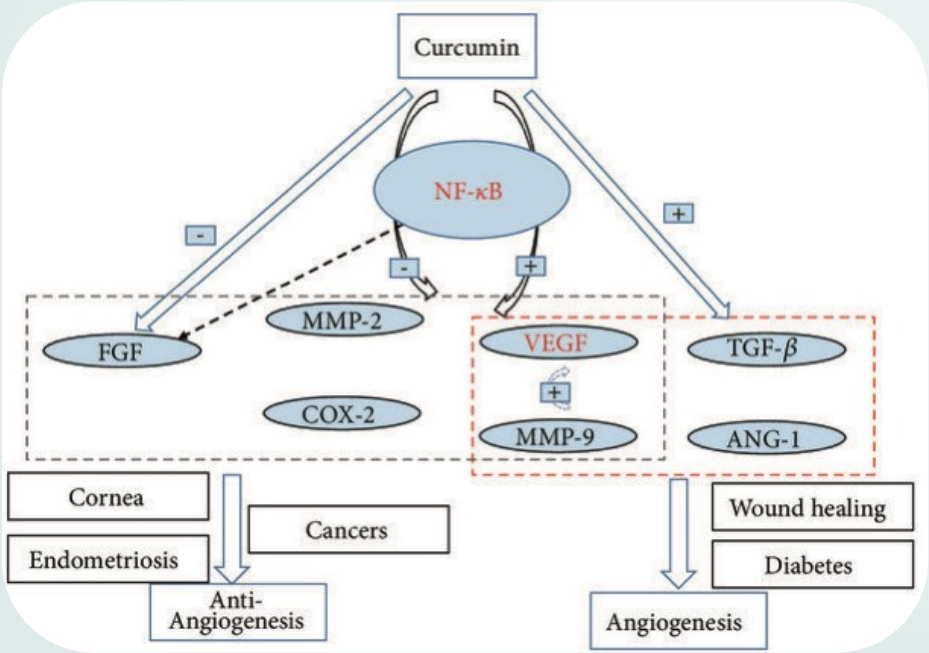
FGF

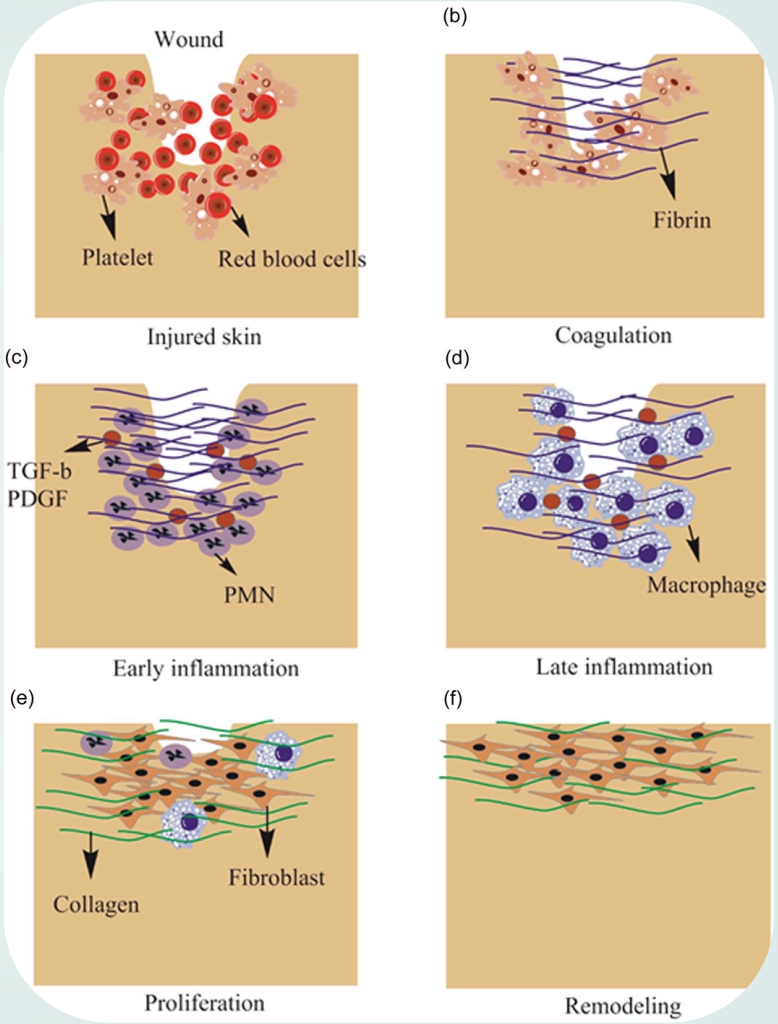
Angiogenesis

- Extensive research on CUR has shown that the keto–enol–enolate form of the heptadienone moiety plays a crucial role in the anti–oxidant activities of curcumin.
- In acidic and neutral conditions the bis–keto form acts as a potent proton donor while at pH > 8, the enolate form predominates and curcumin acts as an electron donor.
- The presence of enolate in solution is found to be important in the radical–scavenging ability of curcumin.
- Acidic environment is known to promote angiogenesis.



- Curcumin enhances the expression of TGF-beta1 which improves angiogenesis.
- Dextran hydrogel containing curcumin-loaded poly(lactide)/poly(ethylene glycol) nanomicelles applied to a dermal wound accelerated angiogenesis, fibroblast accumulation, and wound healing.





Thank you for your attention!

References

1. Govardhanagiri, S., Bethi, S., & Nagaraju, G. P. (2019). Small Molecules and Pancreatic Cancer Trials and Troubles. Breaking Tolerance to Pancreatic Cancer Unresponsiveness to Chemotherapy, 117–131.
2. Yi, B., Dissanayaka, W. L., & Zhang, C. (2020). Growth Factors and Small-molecule Compounds in Derivation of Endothelial Lineages from Dental Stem Cells. Journal of Endodontics, 46(9), S63–S70.
3. Goonoo, N., & Bhaw-Luximon, A. (2019). Mimicking growth factors: role of small molecule scaffold additives in promoting tissue regeneration and repair. RSC Advances, 9(32), 18124–18146.
4. Mitchell J and Lo KWH (2022), Small molecule–mediated regenerative engineering for craniofacial and dentoalveolar bone. Front. Bioeng. Biotechnol. 10:1003936.
5. Lo, K.W.-H. *et al.* (2014) "Small-molecule based musculoskeletal regenerative engineering," *Trends in Biotechnology*, 32(2), pp. 74–81. Available at: https://doi.org/10.1016/j.tibtech.2013.12.002.
6. Aravamudhan A, Ramos DM, Nip J, Subramanian A, James R, Harmon MD, Yu X, Kumbar SG. (2013). Osteoinductive small molecules: growth factor alternatives for bone tissue engineering. Curr Pharm Des. 9(19), :3420–8.
7. Lanza, R.P. et al. (2020) Principles of Tissue Engineering. London: Academic Press, an imprint of Elsevier.
8. Marx, D. *et al.* (2020) "A review of the latest insights into the mechanism of action of strontium in Bone," *Bone Reports*, 12, p. 100273. Available at: https://doi.org/10.1016/j.bonr.2020.100273.
9. Efe, J.A. and Ding, S. (2011) "The evolving biology of small molecules: Controlling cell fate and identity," *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1575), pp. 2208–2221. Available at: https://doi.org/10.1098/rstb.2011.0006.
10. Laurencin, C.T. *et al.* (2014) "Delivery of small molecules for Bone Regenerative Engineering: Preclinical Studies and potential clinical applications," *Drug Discovery Today*, 19(6), pp. 794–800. Available at: https://doi.org/10.1016/j.drudis.2014.01.012.
11. https://www.fda.gov/