Table 2. In *vitro* characterization of PEG_{400}-OA and $mPEG_{2000}$-OA formulation of curcumin using Dynamic Light Scattering and TEM/AFMmicroscopy

Dendrosome + Curcumin	Morphology	PDI	%E.E	Size(nm)	ζ-potential	Ref.
PEG_{400}/OA + Curcumin	Spherical	0.4± 0.03	87.65±1.62	142.97±4.27	-7.8±1.82	(14)
Technique of evaluation	TEM	DLS	Dialysis-HPLC	DLS and TEM	DLS	
$mPEG_{2000}$/OA+ Curcumin	Spherical	0.47±0.071	87.1±7.7	18.33±5.32-99.4±65 *	-32.6± 11.1	(40)
Technique of evaluation	AFM	DLS	Spectroscopy	DLS and AFM	DLS	

*Size was different based on the micelle (18nm) or polymerosome(99.4nm) formation

Tracking of curcumin uptake into the cells by dendrosome based on native fluorescent property of curcumin

Native fluorescence property of curcumin provides simple way to analyze curcumin entrance using fluorescent microscopy. In Figure 7a green florescence shows curcumin uptake facilitated by dendrosome; PEG_{400}-OA/cur (curcumin encapsulated in PEG_{400}-OA) into the WEHI-164 cells. Additionally, $mPEG_{2000}$-OA improves uptake of curcumin in U87MG cells (15, 25). Our results show that dendrosomes increased the curcumin water solubility and facilitate the uptake of curcumin into the U87MG glioblastoma cancer cells Figure 7b.

Evaluation of anti-cancer properties of dendrosomal nanocurcumin

Anticancer efficacy of dendrosomal nanocurcumin was considered on different human and mouse cancer cells in *vitro* and *vivo*. All of these experiments showed that dendrosomal curcumin efficiently suppressed cancer cells in comparison with naked curcumin. Besides, no cytotoxicity was connected to dendrosome alone. This suggests that dendrosome only increases the water solubility of curcumin. In a dose-dependent experiment, acute and chronic cytotoxicity of nanocurcumin has evaluated by Alizadeh *et al*. in mice model of BALB/c. All aspects including blood chemicals, hematological parameters, inflammatory responses, liver and kidney function were considered during one week of consecutive injection. It is found that nanocurcumin is a safe formulation even at dose of 31mg/kg, and in higher concentration can be observed (28).

Additionally, we found that dendrosomal curcumin follows the dose- and time-dependent manner in this way. Table 3 reviewed all of the examined cells in which dendrosomal curcumin was effective. The first report on anticancer property of dendrosomal curcumin was published by Babaei *et al.* where the anticancer potential of dendrosomal curcumin was investigated on mouse model of Fibrosarcoma in vitro and vivo (23).

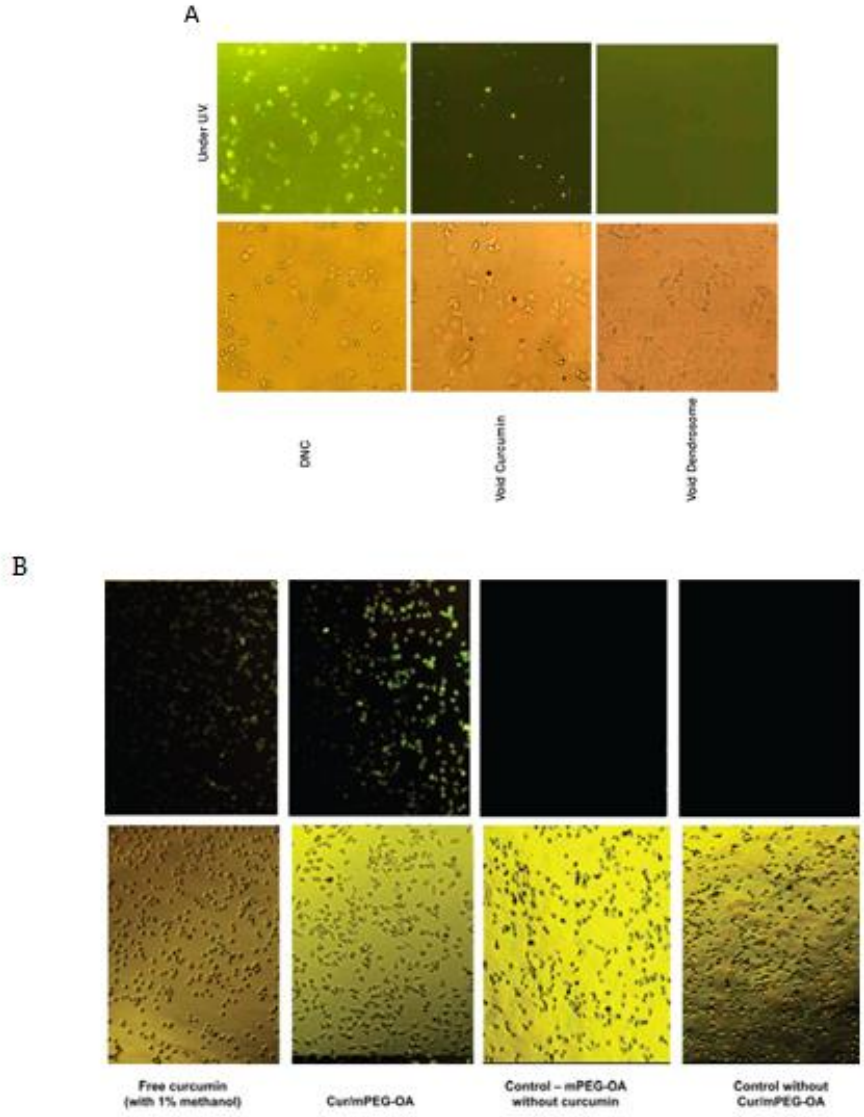

Figure 7. Tracking the uptake of curcumin into the A) WEHI-164 and B) U87MG(40) cancer cells based on native florescent character of curcumin; 8 hours after treatment with DNC, void curcumin and void dendrosome. Above, cells under U.V. spectrum and below side shows the same cells from the same view under visible spectrum.

Data obtained from in vitro experiments demonstrated that dendrosomal curcumin inhibited A431 and WEHI cells at concentration of 5-20 μM in a time- and dose-dependent manner. It seems that induction of apoptosis in a caspase dependent way as well as PARP cleavage is the reason of this inhibitory property. Interestingly, administration of 12.5mg/kg dendrosomal curcumin significantly decreased the tumor size in BALB/C mice model of fibrisarcoma following increasing of survival (Fig. 8). Consequently, it is found that dendrosomal curcumin stimulate the INF-γ production. Therefore, it is postulated that anti-tumor immunity caused by dendrosomal nano-curcumin may involve in tumor removal (23).

Table 3. Effective concentration (LD50) of dendrosomal curcumin preparation on different mouse and human cell lines using MTT assay after 24h drug exposure

Cell line	Origin	Type	Type	24h- LD50 (µM)	48h- LD50 (µM)	Ref.
Wehi	Mouse	Cancerous	Fibrosarcoma	16.8	7.5	(23)
A431	Mouse	Cancerous	Epidermal carcinoma	19.2	14.3	(23)
4T1	Mouse	Cancerous	Breast	35	25	(37)
U87MG	Human	Cancerous	Glioblastoma	20	20	(14)
5637	Human	Cancerous	Bladder	20	15	(24)
HepG2	Human	Cancerous	Hepatocelular carcinoma	30	23	(32, 33)
Huh-7	Human	Cancerous	Hepatocelular carcinoma	30	21	(32, 33)
AGS	Human	Cancerous	Gastric adenocarcinoma	13	7.5	(41)
HFSF-PI3	Human	Normal	Fibroblast	25	25	(14)
MEF	Mouse	Normal	Fibroblast	>45	>45	(42)
hMSC	Human	Normal	Bone-marrow derived Mesenchymal stem cells	28	30	(14)

Figure 8. Dendrosomal curcumin decrerase the tumor size in mice model of fibrosarcoma. A) Mouse with fibrosarcoma tumors, B) Mouse treated with dendrosomal curcumin at day 36

The inhibitory role of dendrosomal curcumin was evaluated in azoxymethane-induced colon cancer mice model. Similar to fibrosarcoma models, dendrosomal curcumin decreased the tumor size in mice in comparison with non-administrated control group. Additionally, dendrosomal curcumin resulted in reduction of nuclear/cytoplasmic ratio, epithelial stratification and nuclear dispolarity in histological staining of tissue taking from sacrificed mice. It has also confirmed that Bax/Bcl2 ratio was decreased following DNC treatment showing the activation of apoptotic pathway (23). In Glioblastoma cells, master genes of proliferation pathways including *Oct4*, *Sox-2* and *Nanog* were significantly suppressed by dendrosomal curcumin leading to induction of apoptosis in these cells. In parallel, *miR-145*- the negative regulator of *Oct4*, *Sox-2* and *Nanog*- was upregulated after DNC treatment. Therefore, DNC indirectly downregulated proliferation genes via *miR-145* induction (14, 29).

Table 4. Genes and Molecular pathways targeted by dendrosomal curcumin preparation

Genes	Origin	Cancer cells	Up/down	Molecular methods	Ref.
OCT4 A			Down	Realtime PCR	(14, 24)
OCT4B1		Brain and bladder	Down	Realtime PCR	(14, 24)
SOX2	Human		Down	Realtime PCR	(14, 24)
Nanog			Down	Realtime PCR	(14, 24)
miR-145			Up	Realtime PCR	(14)
DNMT1			Down	RT-PCR	(32)
DNMT3A			Down	RT-PCR	(32)
DNMT3B			Down	RT-PCR	(32)
hTERT			Down	RT-PCR	(33)
TGFβ1	Human	Hepatocellular carcinoma	Up	RT-PCR	(33)
Smad3			Up	RT-PCR	(33)
Smad7			Up	RT-PCR	(33)
MEG3			Up	RT-PCR	(33)
miR-29a			Up	Realtime PCR	(32)
miR-185			Up	Realtime PCR	(32)
CAT			Up	Realtime PCR	(39)
HO-1	Human	hMSC	Up	Realtime PCR	(39)
VEGF			down	Realtime PCR	(37)
NFkB	Mouse	Breast	down	Realtime PCR	(37)
MMP-9			down	Realtime PCR	(37)
BAX			up	Realtime PCR	(42)
BCL2			down	Realtime PCR	(42)
VEGF	Mouse	Fibrosarcoma	down	Realtime PCR	(42)
MMP9			down	Realtime PCR	(42)
COX-2			down	Realtime PCR	(42)

As the targeting of proliferation pathways is one of the suggested ideas in blocking the cancer cell (30), this potential of dendrosomal curcumin is valuable although further in *vivo* confirmation is urgent. Similarly, dendrosomal curcumin induced apoptosis by suppression of pluripotency genes in 5637 bladder cancer cells although this downregulation was *miR-145*-independent (24, 29). Dendrosomal curcumin induced DNA hypomethylation resulting in re-expression of a silenced tumor suppressor *MEG3*. Due to promoter hypermethylation, the expression of this long non-coding RNA is downregulated in hepatocellular carcinoma. Interestingly, it is found that dendrosomal curcumin downregulates DNA methyl transferases including *DNMT3A*, *DNMT3B* and *DNMT1* by upregulation of *miR-29a* and *miR-185,* respectively, and reverse the epigenetic signature of cancer cells (31, 32). In Huh-7 hepatocellular carcinoma cells, dendrosomal curcumin also inhibited the promoter of telomerase gene (*hTERT*) through the induction of TGFβ signaling pathway. The genes *TGFβ*, *Smad3* and *Smad7* were upregulated after 72h post-treatment with 15μM dendrosomal curcumin. Similarly, hTERT was downregulated in AGS gastric adenocarcinoma cells following DNC treatment (33). As previously reported, telomerase is upregulated in most of the cancer cells leading to uncontrolled proliferation (34). Additionally, anti-apoptotic gene *survivin* was downregulated with DNC in AGS lines. The upregulation of apoptotic gene Bax also confirmed in these cells following treatment (35, 36). It has also demonstrated that nanocurcumin decreased the tumor size of mouse model of breast

cancer through downregulation of angiogenic and metastatic genes. The expression profile of *VEGF, MMP-9* and *NFκB* were downregulated after treatment with dendrosomal curcumin showing the anti-angiogenic and metastatic potential of this nano preparation (37). The inhibitory role of nanocurcumin on extracellular matrix of SW40 cell line of colorectal has been also demonstrated (38). Adversely, we found that dendrosomal curcumin can also act as a protective agent at low concentration. In such doses, dendrosomal curcumin decreases the ROS production and lipid peroxidation protecting the cells from oxidant damages. Furthermore, upregulation of catalase (*CAT*) and hemoxygenase-1 (*HO-1*) gene expression following DNC treatment is consistent with anti-oxidant role of this nano formulation (39). Therefore, it seems that concentration of dendrosomal curcumin determine that which pathway; anticancer or anti-oxidant can activate in target cells. All of these data confirmed the high potency of dendrosome nanocarriers to improve the anticancer potential of curcumin by increasing the solubility and cellular bioavailability of hydrophobic curcumin in aqueous media.

Evaluation of toxic effects of dendrosomal curcumin on normal cells

Regarding to cytotoxic side effects of most of anticancer drugs on normal cells, the cytotoxic properties of dendrosomal curcumin was also investigated in different normal cells to answer if DNC has inhibitory effects on these cells. Interestingly, it is found that DNC had no inhibitory properties on normal cells or inhibit normal cells in a concentration higher than the dose effective on cancerous one (14, 23). These observation confirmed that dendrosomal curcumin preferentially target cancer cells than normal counterparts and can be considered as a promising agent in cancer therapy.

Conclusion

Here, we aimed to review a novel generation of nanocarrier termed dendrosome which is produced in our research group. We demonstrated that these biocompatible polymeric micelles/ polymersomes not only have no cytotoxicity on normal cells but also efficiently suppress tumor cells in *vivo* and *vitro*. Inhibitory effects of dendrosomal curcumin were only mediated by curcumin and dendrosome only play a role as a delivery vehicle. Physical characteristics of dendrosomes showed that they are spherical and have relatively high encapsulation efficiency and drug loading capacity and additionally high stability over time. CMCs of dendrosomes are relatively in a good and acceptable range for a micelle family, although designing novel heavier di or tri-block nanocarriers are recommended with bigger hydrophobic and hydrophilic parts which will create nanocarriers with even lower CMC and more stability specially when they administrate and expose to extremely diluted condition of real blood circulation. Considering the results, dendrosome family has appropriate properties which make them good candidates for drug and small nucleic acid delivery. Although, there is a long way to finalize this formulation as a drug in pharmacy, our results until now confirmed nanocurcumin can be considered as a relatively safe formulation for cancer therapy.

Perspective

Recent year's combination therapy has received a lot of attention for treating chronic diseases such as cancers. This review study recommends the use of dendrosome as a nanocarrier for drug and gene (small

molecule nucleic acids) therapy together. Simultaneous delivery of curcumin and siRNA can be an effective approach for facing common problem of drug resistance in cancer, and application of dendrosome for such researches are highly recommended.

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Disclosure

The authors report no conflicts of interest in this work. .

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