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Review

Collagen-Binding Nanoparticles: A Scoping Review of Methods and Outcomes

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Abstract: (1) Background: Collagen is the main component of the connective tissue, playing an important role in the histological architecture and function of living organisms. Targeted therapy and improved imaging diagnosis can be obtained through collagen-binding nanoparticles that concentrate in the extracellular matrix. (2) Methods: We performed a scoping review of studies that analyzed the binding capacity of collagen-targeting nanoparticles. The search algorithm and inclusion criteria were based on PRISMA and ARRIVE guidelines. (3) Results: Fourteen studies matched all the inclusion criteria. All studies analyzed the distribution of nanoparticles in the collagen matrix, either by using collagen-targeting nanoparticles or by using unmodified ones. Most studies used collagen-binding nanoparticles for vascular research to target sites of endothelial injury, atherosclerotic plaques, or myocardial infarction. Two studies targeted the exposed collagen in models of liver fibrosis. (4) Conclusions: Our review summarizes the current literature on the methods and outcomes of using nanoparticles to target collagen. The studies reveal that there is high applicability for collagen-binding nanoparticles in cardiac or hepatic pathology and they could prove useful for targeted therapy of neoplastic lesions, which show an abundance of stromal collagen.

Keywords: collagen; nanoparticles; theranostics

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1. Introduction

In medicine, the application of nanotechnology is mostly focused on the use of nano-objects (e.g., nanoparticles) to target specific surface peptides for delivering drugs, enhancing their biodistribution, and providing accurate in vivo imaging and sensing [1–5]. The biodistribution of nanoparticles (NPs) must be clearly understood before aiming to target peripheral molecules because once administered in the bloodstream they face important barriers that need to be considered. Firstly, NPs cannot cross the endothelium of healthy vessels. Only where significant inflammation is present and the endothelial junctions widen, or between the fragile, tortuous gaps of tumoral vessels can NPs penetrate [6]. This property is highly exploited in the targeted NPs that are used for drug delivery in tumoral tissues, thus reducing their systemic adverse effects. Another barrier is the reticuloendothelial system (RES) which removes the NPs from circulation, concentrating them in the lymph nodes, spleen and liver [6,7]. To avoid this, polyethylene glycol (PEG) is usually attached to the NPs to increase their blood circulation half-life [8]. Another way to avoid the macrophage pathway is to use small NPs (10–30 nm), as larger NPs (~100 nm) are easily removed from the circulation by the RES [6]. After penetrating the endothelium,

NPs face the interstitial space. Here in the dense collagen fibers, NPs accumulate and, due to the increased stromal component of the inflamed or neoplastic tissues, NPs concentrate even more. Although this is a barrier that prevents NPs from reaching tumoral cells [9], this property can be used to our advantage as the increased accumulation of NPs in the extracellular matrix (ECM) of tumors facilitates the targeted release of drugs, imaging modalities and radiation focusing on the tumor stroma.

Collagen is the main component of the ECM and the primary protein in the living organism [10,11]. Its synthesis is up-regulated during the inflammatory and the remodeling phases of wound healing, and in the tumor microenvironment, imbalances in the collagen architecture is a key factor in the migration and the metastasis of malignant cells [12–14]. The abundance of the collagen fibers in the pathological tissues can be used as a potential target for NPs and herein we aim to analyze the current outcomes of using collagen-targeting NPs (CTNPs) and to ascertain if collagen can be used as a reliable ligand for NPs. This scoping review aims to map and compare how NPs were used to target collagen and to open the future to the possibility of using collagen-targeting NPs as a platform for drug delivery and cancer theranostics.

2. Materials and Methods

2.1. Literature Search and Study Selection

A systematic search of the PubMed and the EMBASE databases was performed for all of the published studies on collagen-targeting nanoparticles using the following search algorithm: collagen AND target OR binding AND nanoparticles. The systematic search was done by adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines which were adapted to the experimental studies. The PRISMA checklist was followed to conduct the methodology (Figure 1). All experimental studies that were published in English from the 1 of January 2011 to the 1 of September 2021 and that described the methods of using nanoparticles to target or accumulate in collagen were triaged for full-text review. Inclusion criteria were used according to the Problem/Population, Intervention, Comparison and Outcome (PICO) formula. The experimental lot (population) consisted of experimental models of collagen deposition. Both in vitro and in vivo models were included. The intervention was defined as the administration of nanoparticles to target or accumulate in the collagen-rich matrix. The comparison criteria were further selected from subgroups of the included studies where applicable control groups were analyzed.

2.2. Data Analysis

The following metadata regarding each of the included studies were extracted: the author name, year of publication and type of study (in vitro or in vivo), as well as the experimental model, the animal model, the tumor cell line, the type of NPs, the protocol of NP delivery and the delivery method that were used in the experiments, and the follow-up of outcomes including immunohistochemistry studies, hematological studies and histology analysis.

2.3. Quality Assessment

Two authors (S.M. and S.I.) independently examined the title and abstract of citations, and the full texts of potentially eligible studies were obtained; disagreements were resolved by discussion. The ARRIVE guidelines were used to quantify the quality of the included studies as previously published [15,16] (Figure 2). Each study was marked for every ARRIVE item with 0 if the data were lacking, 1 if the data were incomplete and 2 if the data were complete. The reference lists of retrieved papers were further screened for additional eligible publications.

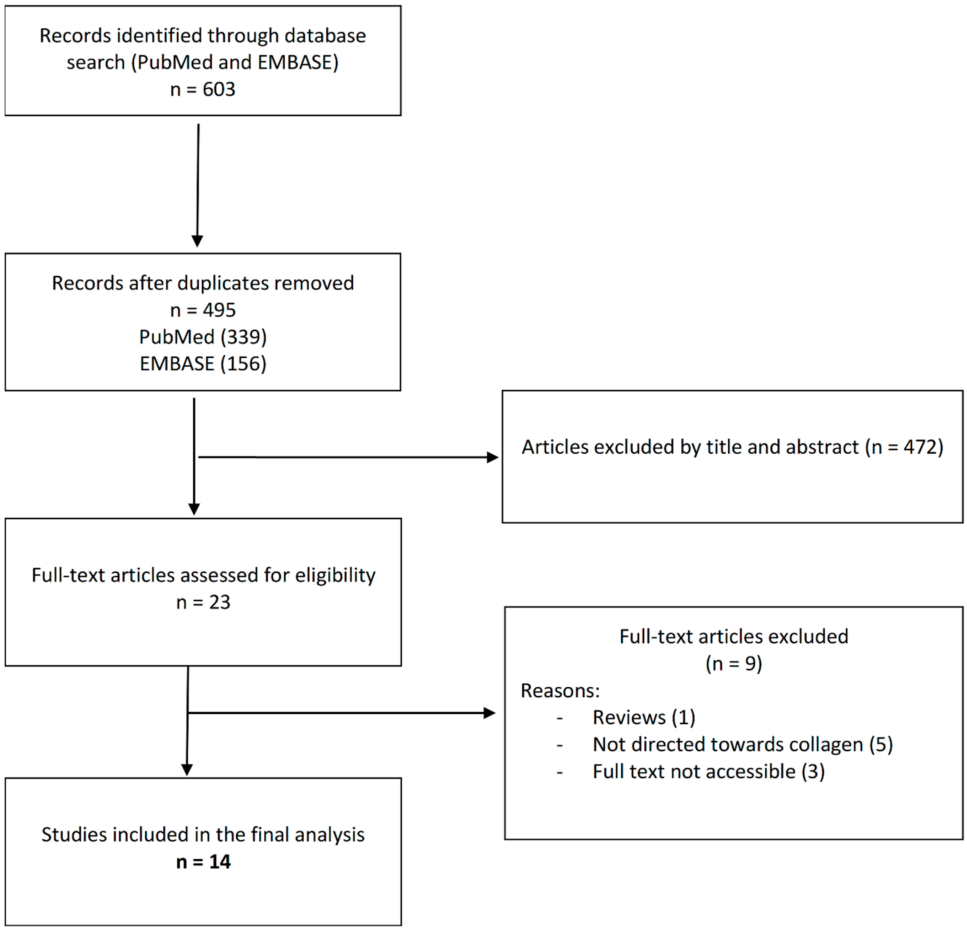

Figure 1. PRISMA flow-chart.

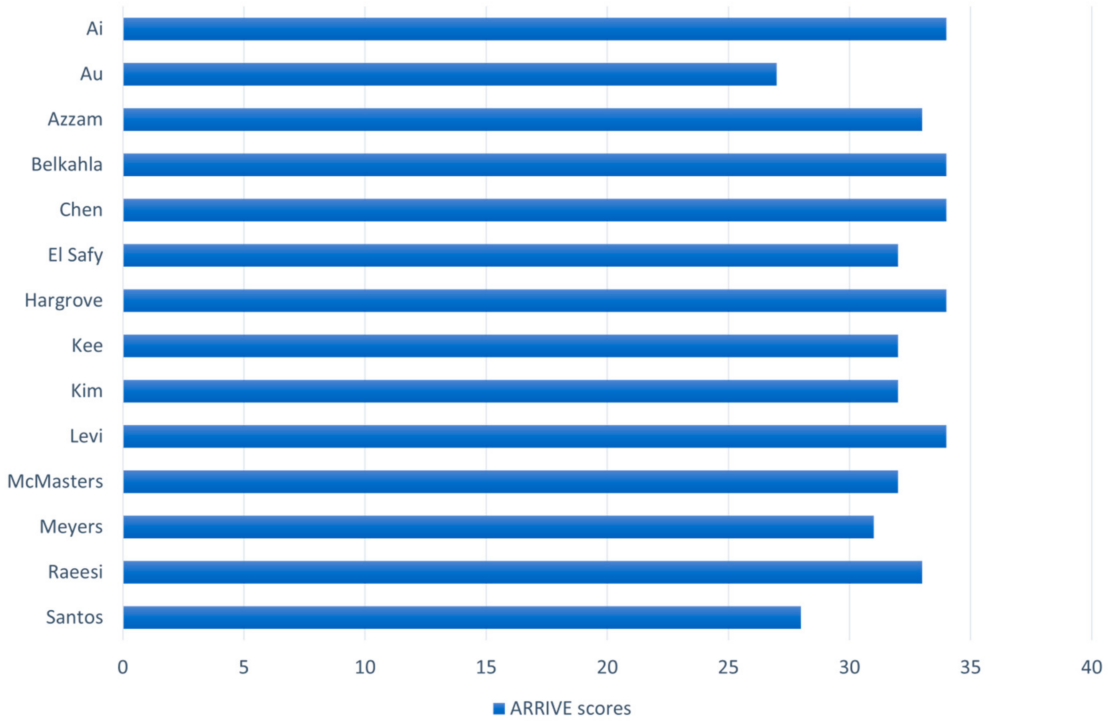

Figure 2. ARRIVE scores.

3. Results

3.1. Literature Review and Design of Eligible Studies

Fourteen studies were selected based on the inclusion criteria [17–30] (Figure 1). The initial search found 603 manuscripts, out of which 108 were duplicates and were removed. Out of the remaining 495 studies, twenty-three full-text manuscripts were assessed for eligibility. All manuscripts were published in the last ten years.

3.2. General Characteristics of Included Studies

All of the included papers studied the distribution of NPs in the collagen matrix, either by using CTNPs or by using unmodified control NPs (cNPs), given their natural tendency to attach to collagen. The models used in the included studies were heterogeneous. Most studies used collagen-binding nanoparticles (CBNPs) for vascular research to target sites of endothelial injury [18,26,28], atherosclerotic plaques [21,25] or myocardial infarction [24] (Table 1). Two studies targeted the exposed collagen in models of liver fibrosis [19,22] and one study used CBNPs to target the articular collagen in osteoarthritic models [17]. There were eleven in vitro experiments and ten in vivo studies (Table 1). The heterogeneity of the experiments proves the versatility of NPs to target collagen at various sites.

Table 1. Overview of the included studies.

First Author	Year	Type of Study	Animal Used	Type of NP	Novelty
Ai [17]	2020	in vitro/in vivo	mice	PEGylated lipid NPs coated with collagen-binding peptides (CBPs)	using CBNPs to reduce cartilage destruction in OA model
Au [18]	2015	in vitro/in vivo	mice	PEGylated NPs coated with collagen IV binding peptides	using CBNPs to target radiotherapy induced endothelial injuries
Azzam [19]	2020	in vitro/in vivo	mice	chitosan NPs	using CBNPs to deliver siRNA to fibrotic liver and reduce profibrogenic gene expression
Belkahla [20]	2020	in vitro	NA	PEGylated USPIO NPs coated with Collagelin	using CBNPs to enhance MRI diagnosis of fibrosis
Chen [21]	2013	in vitro/in vivo	mice	PEGylated HDL NPs coated with CBP (EP3533)	using MRI traceable CBNPs to monitor atherosclerotic plaque changes
El Safy [22]	2020	in vitro/in vivo	mice	chitosan NPs coated with collagenase and CBP (CCQDSETRTFY)	using CBNPs to target and digest collagen rich liver fibrosis
Hargrove [23]	2020	in vitro/in vivo	mice	MCM-41 type mesoporous silica nanoparticles	accumulation of NPs in the stroma of 3D spheroids and peritoneal tumor xenografts
Kee [24]	2018	in vivo	Sprague-Dawley rats	PEGylated AuNPs coated with CBP (CNA35)	using CBNPs to target myocardial infarction scar
Kim [25]	2018	in vivo	mice	chitosan-iron oxide NPs coated with cRGD or collagen IV binding peptides	using CBNPs to target atherosclerotic plaques
Levi [26]	2020	in vitro/in vivo	mice	PLGA NPs coated with GPVI (collagen-binding peptides)	using CBNPs to target endothelial injuries
McMasters [27]	2017	in vitro	NA	polymeric NPs coated with collagen I binding peptides (SILY)	using CBNPs to target endothelial injuries and supress local inflammation
Meyers [28]	2017	in vivo	Sprague-Dawley rats	PEGylated AuNPs coated with CBPs	using CBNPs to target endothelial injuries
Raeesi [29]	2016	in vitro	NA	AuNPs	using heat generating NPs to denaturate collagen and improve diffusion in tumor stroma
Santos [30]	2014	in vitro	NA	polymeric NPs coated with CBP	using CBNPs to target collagen in corneal tissue

PEG = polyethylene glycol; OA = Osteoarthritis; CBNPs = collagen-binding nanoparticles; siRNA = small interfering RNA; USPIO = Ultra Small Super Paramagnetic Iron Oxide Nanoparticles; CBP = collagen-binding peptide; cRGD = Arginylglycylaspartic Acid; PLGA = poly(lactic-co-glycolic acid); MRI (magnetic resonance imaging); AuNPs = gold nanoparticles.

3.3. Nanoparticles: Types and Synthesis Protocols

By a large margin, the bottom-up precipitation synthesis of NPs is the most common technique that was used, according to our analysis, and it reflects the wider literature. The average size of the NPs was 88.3 nm (13–280 nm, Table 2). Polymeric NPs were the most common type as they are easy to elaborate, have good biocompatibility and can be coupled with many other polymers and peptides, which increases their versatility. Most CBPs are polymeric in nature and can be easily coupled to NPs via PEG surface chains. The coupling of CBPs to NPs is the key to actively targeting collagen in the tissues where it is overexposed or overexpressed. Two studies did not use CBPs to increase NPs collagen-targeting sensitivity [23,29], instead the targeting was passive and based on the affinity of the cNPs. Instead of using CBPs, some authors analyzed the intrinsic ability of NPs to accumulate in the extracellular matrix. To further increase the penetration of cNPs in the stroma, some authors chose to thermally denature collagen. Interestingly, Hargrove et al. [23] showed a lower concentration of mesoporous silica NPs in the disrupted collagen. This contradicts the results of Raeesi et al. [29]. An advantage of polymeric NPs is that fluorescent peptides (e.g., cyanine dye) can be conjugated to the polymeric chains, and such NPs were used for the in vivo fluorescent imaging of atherosclerotic plaques [24] and peritoneal tumors [22]. Another common choice is AuNPs. Similarly, to polymeric ones, AuNPs are highly biocompatible and have unique optical and thermal properties, making them MRI traceable and, additionally, they can focus radiant or heat waves at the targeting site. For this reason, Raeesi et al. [29] used AuNPs as they could generate heat and denature collagen, thereby improving the diffusion of the NPs in the tumor extracellular matrix, which was an effective technique to increase the concentration of NPs in the targeted tissue, enhancing the penetration of small (50 nm) and bigger (120 nm) NPs into the thermally treated tissue compared to the untreated one. Ai et al. [17] used ultra-small lipid NPs (~25 nm) in their osteoarthritis model as they have been shown to better penetrate cartilage than other larger NPs.

Table 2. Nanoparticle types.

First Author	Type of NPs	Targeting Type	CBPs	NPs Size (nm, Mean)
Ai [17]	DSPE-PEG dissolved in DMSO + PLGA-COOH (50/50 ratio)	active	WYRGRLC	25
Au [18]	fluorescent rhodamine B-PEG-PLGA NPs autoprecipitation method	active	collagen IV binding peptide	83
Azzam [19]	Chitosan NPs-MO-PDGFR binding peptide	active	Chitosan intrinsic binding	110
Belkahla [20]	USPIO-PO-PEG	active	collagelin	24.5
Chen [21]	DSPE-PEG-COOH-HDL NPs linked to gadolinium		EP-3533	10
El Safy [22]	Chitosan NPs-Collagenase-PEG	active	CCQDSETRTFY	90
Hargrove [23]	MSN-Cy5.5; MSN-Cy5.5-PEG, MSN-Cy5.5-FA	passive	Not used	280
Kee [24]	AuNPs-PEG	active	CNA35	75
Kim [25]	DA-PF 127-Chitosan-IONPs-Cy5.5	active	cRGD; collagen IV binding peptide	77
Levi [26]	AuNPs-PLGA	active	GPVI	243
McMasters [27]	pNIPAM NPs	active	SILY	Not declared
Meyers [28]	Citrate stabilized PEG-AuNPs	active	H2N-KLWVLPK-COOH	13
Raeesi [29]	AuNPs	passive	Not used	50; 120
Santos [30]	PFBT polymeric NPs autoprecipitation method	active	collagen IV binding peptide	30

DSPE = distearoyl phosphatidyl ethanolamine; DMSO = dimethyl sulfoxide; PLGA = poly(lactic-co-glycolic acid); MO = fluorescent model oligonucleotide; USPIO = Ultra Small Super Paramagnetic Iron Oxide Nanoparticles; PO = Phosphonate; MSN = mesoporous silica nanoparticles; Cy5.5 = cyanine 5.5; FA = folic acid; IONPs = Iron oxide nanoparticles; DA-PF 127 = diacrylate pluronic F127; GPVI = collagen receptor glycoprotein VI; pNIPAM = Poly(N-isopropylacrylamide); PFBT = Poly(fluorene-alt-benzothiadiazole); PDGFR = Platelet-derived growth factor.

3.4. Outcomes of CBNPs

Most types of NPs (polymeric, lipid, metallic) showed increased collagen accumulation once they were functionalized with collagen-targeting peptides (Tables 3 and 4). Linkers, such as PEG, were used to attach collagen binders to the NPs (Table 2). Fluorescent peptides were used in most cases in order to quantify the retention of NPs. Even if they were not linked to CBPs, some NPs (e.g., chitosan, gold) possessed an intrinsic capacity to attach to the collagen fibrils in one study [21]. Control chitosan NPs showed similar binding capacities compared to CBNPs. Compared to chitosan, AuNPs have photothermal properties and can concentrate heat when bound to collagen, thus disrupting and exposing more collagen fibrils. The denaturation of collagen was shown to increase the affinity of NPs by Raeesi et al. [28]. This was not the case in Hargrove's experiments [22]. In their ovarian adenocarcinoma xenografts model, mesoporous silica nanoparticles (MCM-41) showed less penetration in the tumor stroma when collagen was disrupted.

Table 3. In vitro models.

First Author	Experimental Model	Route of Administration	Evaluation of NPs Distribution	Outcomes
Ai [17]	C57BL/6 mice femoral heads	DID-CBNPs versus DID-cNPs were incubated with the femoral heads for 24 h	Fluorescence Micropscopy	two-fold increased accumulation of CBNPs compared to cNPs
Au [18]	collagen IV coated well plate	incubation of CBNPs vs. cNPs	Fluorescence Spectroscopy	tenfold increased accumulation of CBNPs compared to cNPs
Azzam [19]	hepatic stellate cell lines GRX and HEK293	incubation chitosan NPs and chitosan-PDGFR binding peptide NPs	Fluorescence Spectroscopy	increased accumulation of NPs. No control group
Belkahla [20]	Collagen I hydrogel (from rat tail tendon)	incubation of free Collagelin vs. Collagelin-NPs vs. cNPs	Histology (Prusian Blue stain)	two-fold increased accumulation of CBNPs compared to cNPs
Chen [21]	collagen well plates	incubation of CBNPs vs. cNPs	Fluorescence Microscopy	fourteen-fold increased accumulation of CBNPs compared to cNPs
El Safy [22]	collagen well plates	incubation of CBNPs vs. cNPs	Fluorometry	no significant difference between CBNPs and cNPs
Hargrove [23]	ovarian adenocarcinoma 3D tumor spheroid	incubation of non-targeted NPs	Fluorescence Micropscopy	increased accumulation of NPs. No control group
Levi [26]	tubular stenosis model coated with collagen	1 hour circulation of CBNPs vs. BSA-NPs through tube	Fluorescence Micropscopy	twenty-fold increased accumulation of CBNPs compared to cNPs
McMasters [27]	human coronary artery proliferative smooth muscle cells	24 h incubation	Fluorescence Micropscopy	increased accumulation of NPs. No control group
Raeesi [29]	bovine collagen I solid matrix	incubation of non-targeted AuNPs	Transmision Electron Microscopy	Collagen denaturation through hyperthermia improves AuNPs retention
Santos [30]	mouse corneal tissue	incubation of CBNPs	Fluorescence Micropscopy	Increased retention of CBNPs in the corneal collagen-rich stroma

DID = 1,10-dioctadecyl-3,3,30,30-tetramethylindodicarbocyanine Perchlorate; PDGFR = Platelet-derived growth factor; BSA = bovine serum albumin; cNPs = control nanoparticles.

Table 4. In vivo models.

First Author	Experimental Model	Route of Administration	Evaluation of NPs Distribution	Outcomes
Ai [17]	CIOA mouse model-intra-articular (knee) collagenase injections	intra-articular injections of CBNPs vs. cNPs	histologic analysis (H&E and safranin-O stains)	42% retention of CBNPs compared to 18% of cNPs
Au [18]	RT induced vessel injury (left flank exposed to single high-dose RT 30Gy)	tail vein iv injection of CBNPs and cNPs	fluorescent imaging and histology analysis	six fold increased accumulation of CBNPs compared to cNPs
Azzam [19]	Carbon Tetrachloride (CCl4) model of liver fibrosis	tail vein iv injection of CBNPs	histologic analysis (H&E stain)	increased accumulation of CBNPs in the fibrotic liver, not in healthy liver
Chen [21]	aortic atherosclerotic plaques	tail vein iv injection of CBNPs and cNPs	MRI	80% increase in CBNPs retention compared to cNPs
El Safy [22]	Carbon Tetrachloride (CCl4) model of liver fibrosis	tail vein iv injection of CBNPs and cNPs	histology analysis	collagenase linked NPs are able to reverse liver fibrosis
Hargrove [23]	peritoneal ovarian cancer xenograft model (OVCAR-8)	intraperitoneal injection of CBNPs	Fluorescence Miscropscopy	increased accumulation of NPs. No control group
Kee [24]	Sprague-Dawley rats with myocardial infarction	tail vein iv injection of CBNPs and cNPs	CT molecular imaging	increased accumulation of CBNPs
Kim [25]	aortic atherosclerotic plaques (Apolipoprotein E knockout mice)	tail injection of CBNPs (cRGD vs. CIVBP)	NIR fluorescence; ex vivo MRI	30% increased accumulation of cRGD-IONPs compared to CIVBP-IONPs
Levi [26]	mice carotid artery partial ligature	tail vein iv injection of CBNPs and cNPs	Fluorescence Miscropscopy	increased accumulation of CBNPs at the stenotic site
Meyers [28]	carotid artery balloon injury model	tail vein iv injection of CBNPs and cNPs	Fluorescence Miscropscopy	increased accumulation of CBNPs compared to cNPs

CIOA = collagen induced osteoarthritis; H&E = hematoxylin & eosin; RT = radiotherapy.

3.5. In Vitro Studies

In most studies (n = 11), the collagen affinity was initially tested in an in vitro experiment. However, the chosen models vary; most researchers used well-plates, in which type IV collagen was cultivated, while others used specific cell lines (e.g., hepatic cell lines, smooth muscle). One study used a spheroid cell culture of an ovarian adenocarcinoma line [23] and one used a tissue sample of a mouse cornea [30], which is highly rich in collagen. In all experiments, CBNPs and cNPs were directly incubated with the experimental cell line and collagen-binding was quantified by using fluorescent imaging. When compared to cNPs, CBNPs showed significantly more retention ranging from a two-fold to a fourteen-fold increase (Table 3).

3.6. In Vivo Studies

Ten in vivo studies were performed in order to assess the NP–collagen interaction. Overall, the NPs exhibited an enhanced adhesion to collagen sites, regardless of the model that was used. The control chitosan NPs possess an intrinsic collagen affinity, and this was demonstrated herein [22]. When bound to CBPs, their attachment to collagen increased significantly. In most cases, the NPs were administered intravenously through the dorsal tail vein. One study studied the delivery of NPs via intra-articular injection [17] and one via peritoneal infusion [23]. Under direct visualization via fluorescent microscopy, MRI or CT (computed tomography), CBNPs were shown to have an increased affinity to the collagen fibrils. When compared to cNPs, CBNPs had at least 30% higher concentrations (Table 4). Collagen targeting was performed by using various peptides, which are shown in Table 2. Azzam et al. [19] showed that different NPs (chitosan NPs and NPs that were tagged with

low- and high-peptide density) presented different outcomes in relation to the targeted tissue. Their results explained that small-sized and hydrophilic NPs accumulate more in the fibrotic liver (with overexpressed collagen in the ECM) than in the healthy equivalent; also, animals with fibrotic livers, which were pre-treated with collagenase, had a 1.7–1.9-fold reduction in the accumulation of chitosan NPs and low-peptide NPs, while the high-density peptides had a tendency to concentrate more in the collagenase-treated fibrotic liver. One study [25] compared two types of CBPs: Arginylglycylaspartic Acid (cRGD) and type IV collagen-binding peptide (C4BP). While both increased the concentration of NPs, cRGD showed a higher affinity. Metallic NPs, especially AuNPs, showed a stable intravascular biodistribution with a slow excretion rate. Kee et al. [24] showed the persistent circulation of AuNPs at six hours post-injection. This is an important step forward compared to the iodine contrast agents which are usually excreted after fifteen minutes. Because of their intravascular stability when injected, Kee et al. [24] were able to observe, via molecular CT imaging, the attachment of CBNPs to the exposed collagen of myocardial infarction scars.

4. Discussion

The major findings of this review are: (i) collagen proved to be a reliable target for NPs in both in vitro and in vivo experiments; (ii) various types of NPs can be attached to collagen-binding peptides via PEG linkers and all of them show an improved retention in collagen; and (iii) most studies on CBNPs are focused on cardiovascular experimental models of atherosclerosis or vascular stenosis.

Nanomedicine is focused on improving the retention of NPs and their diffusion at the targeting site [31]. In the field of oncology, there is an important area of research that is focused on creating functionalized nanocarriers that can attach to the tumor and either release chemotherapeutics, in order to achieve the maximal concentration near the cancer cells, or to act as focusing points for X-rays [9,32–34]. NPs accumulate in the tumor stroma through the wide endothelial gaps of the fragile tumoral vessels [35]. Similarly, NPs accumulate in areas of inflammation where cell-to-cell junctions widen to promote the extravasation of inflammatory cells [36]. After extravasation, NPs reach the tumor microenvironment, which is mostly composed of ECM. We believe that the ECM can potentially be a better targeting point for NPs than neoplastic cells, which are naturally bound to replicate and change their surface peptides considerably. Collagen is the main protein of the ECM and for this reason, we aimed to analyze how collagen was used as a targeting peptide for NPs. We evaluated all of the in vitro and in vivo experiments that used NPs that were linked to different peptides in order to enhance adhesion to collagen fibrils. In our analysis, we found only one study that used NPs that were targeted at the collagen of a tumor experimental model. Most studies were in the field of cardiovascular research and used models of atherosclerosis or endothelial injury. However, all of the studies showed an improved accumulation of NPs when they were linked to CBPs, regardless of the experimental models used or the type and size of the NPs. Interestingly, chitosan NPs have an intrinsic capacity to adhere to collagen, and they may be used without collagen binders, which eases the synthesis. Similarly, unmodified AuNPs were shown to accumulate in the ECM by adhering to collagen. AuNPs have a unique photothermal capacity to generate heat and were able to disrupt the surrounding collagen matrix in an in vitro bovine collagen model [29]. Subsequently, AuNPs showed an improved retention to the exposed collagen fibrils through denaturation. This is an insight into how AuNPs could be used to penetrate through the tumor stroma by thermally disrupting collagen, thereby exposing fibrils and leading to the positive feedback of more nanoparticle accumulation. According to our analysis, both passive and active targeting of collagen was used. Both techniques showed an increased affinity for collagen fibrils, either through their intrinsic binding capacity (e.g., AuNPs, chitosan) or via CBPs that were loaded onto NPs. However, for in vivo studies, one must consider the half-lives of different NPs. cNPs have a low circulation life and this might impede them from reaching their target. CBPs create a shear stress on the NP surface, which aids in the binding of and penetration of NPs into the endothelium. Once in

the interstitial space, CBNPs will adhere where collagen is more abundant, in the tumoral stroma. If one is aiming to deliver chemotherapy to the tumor cell, this will have a barrier effect and the NPs will not reach the cells. Here is where the work of Raessi et al. shows its value: the collagen can be used as the first pillar of fixation where active NPs can bind and disrupt the collagen fibrils, either through enzymatic denaturation or thermal denaturation (if metallic NPs are used), which paves the way for drug-loaded NPs to reach the cells. None of the included studies analyzed the toxicity of NPs in in vivo studies. The systemic effect of NPs is one of the main issues that limits their clinical use. The biodistribution of CBNPs is different than other NPs, as they will accumulate in stroma-rich organs, and this should be analyzed in future studies. While CBNPs showed good affinity for the atherosclerotic plaque, it is not clear if this bond will have a thrombogenic side effect.

5. Conclusions

Our review summarized the current literature on the methods and outcomes of using NPs to target collagen. Overall, CBNPs showed an increased adhesion to collagen regardless of the size or the type of NPs that were used. Chitosan NPs can be used to target collagen without the use of targeting peptides. Most experiments are validated on cardiovascular models and models of liver fibrosis. The versatility of CBNPs should be used in future studies on experimental models of cancer, as the abundance of collagen in the tumor stroma may be a stable ligand for nanocarriers. Targeted drug delivery with CBNPs, in accordance with different carcinogenic tissues, should be the subject of future studies with a focus on how new preparation schemes of NPs could ease the tissue-specific and affinity-based concentrations.

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